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PHARMACOGENETICS

Fluconazole-induced intoxication with phenytoin in a patient with ultra-high activity of CYP2C9

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Abstract
Purpose The cytochrome P450 enzyme CYP2C9 metabolizes several important drugs, such as warfarin and oral antidiabetic drugs. The enzyme is polymorphic, and all known alleles, for example, *CYP2C9**2 and*3, give decreased activity. Ultra-high activity of the enzyme has not yet been reported.
Methods We present a patient with Behçet's disease who required treatment with high doses of phenytoin. When fluconazole, a potent inhibitor of CYP2C9, was added to the treatment regimen, the patient developed ataxia, tremor, fatigue, slurred speech and somnolence, indicating phenytoin intoxication. On suspicion of ultra-high activity of CYP2C9, a phenotyping test for CYP2C9 with losartan was performed.
Results The patient was shown to have a higher activity of CYP2C9 than any of the 190 healthy Swedish Caucasians used as controls.
Conclusions Our finding of an ultrarapid metabolism of losartan and phenytoin may apply to other CYP2C9 substrates, where inhibition of CYP2C9 may cause severe adverse drug reactions.

Keywords Behçet's disease · Losartan · Ultrarapid metabolizer

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Background

The ultrarapid metabolism (UM) of antidepressant drugs, such as amitriptyline and nortriptyline, has long been recognized and documented as a therapeutic problem. A patient with this condition had to be treated with high doses to reach therapeutic concentrations [1]. This condition has been shown to be due to a cytochrome P450 2D6 (CYP2D6) gene duplication [2, 3]. Among Swedish Caucasian patients not responding to antidepressant treatment, the frequency of *CYP2D6* gene duplication was found to be tenfold higher than that in healthy subjects, indicating that UM plays an important role in patients who fail to respond to treatment [4]. *CYP2D6* gene duplication is therefore a significant factor that must be taken into account when prescribing treatment with many of the drugs metabolized by the CYP2D6 enzyme [5].

Two single nucleotide polymorphisms (SNPs) constituting the *CYP2C19**17 allele cause an increased activity of the CYP2C19 enzyme [6]. The increase in the rate of metabolism of drugs, such as citalopram and omeprazole, is, however, relatively small and usually without clinical relevance [7].

A third enzyme, CYP2C9, is also polymorphic, and the two alleles, *CYP2C9**2 and especially *3, encode enzymes with decreased enzyme activity [8]. CYP2C9 metabolizes a number of important drugs, such as phenytoin, warfarin, losartan, oral antidiabetics and non-steroidal anti-

inflammatory drugs (NSAIDs) [9]. Here, we present a patient who, despite receiving high doses of phenytoin, seldom attained the recommended therapeutic concentrations of 40–80 μmol/L [10]. When she received the CYP2C9 inhibitor fluconazole, she became intoxicated with high plasma/serum (P/S) concentrations of phenytoin. We therefore tested the hypothesis that the patient had ultra-high CYP2C9 activity.

Case report

A 59- year-old Swedish woman of Caucasian origin, with a weight of 50 kg, was referred to the Department of Rheumatology at the Karolinska University Hospital, Stockholm, in the mid-1980s due to polyarthritis and recurrent uveitis. She was initially treated with NSAIDs and corticosteroids. In the mid-1990s, the symptoms aggravated with progressive systemic inflammation, skin vasculitis, recurrent oral ulcerations, and intestinal inflammation (ileitis). Histological examination of the skin and intestine revealed pathological changes in accordance with Behçet's disease (BD), and in 1998, the definitive diagnosis was made. The same year she developed recurrent epileptic seizures, and treatment with phenytoin was initiated. She also presented symptoms from several cranial nerves, which were interpreted as peripheral nervous system manifestations of BD. During her long periods of severe and—to a large extent—therapy-resistant disease, she has undergone treatment with virtually all available immunomodulation therapies, including cyclosporine A, sulfasalazine, azathioprine, methotrexate, cyclophosfamide and tumor necrosis factor (TNF)-blockers (infliximab, etanercept). Due to her epileptic seizures she has also been under constant treatment with phenytoin. Despite receiving phenytoin doses that are two- to threefold higher than those recommended in the Swedish physicians desk reference (4–5 mg/kg), she seldom reached therapeutic concentrations, and her compliance with treatment was questioned. The phenytoin concentrations were usually between 10 and 20 μmol/L and often below the level of quantification (10 μmol/L).

In 2000, the patient was referred to the emergency unit due to confusion and vertigo. She was at that time being treated with phenytoin 700 mg daily and fluconazole 50 mg a day, the latter having been introduced 2–3 days earlier due to oral candidiasis. Her central nervous system (CNS) symptoms developed soon after the initiation of the fluconazole therapy. The phenytoin concentration reached a toxic level, 138 μmol/L, and the neurological symptoms were considered to be an adverse drug reaction (ADR) due to an interaction between fluconazole and phenytoin. Laboratory results showed anaemia, hypoalbuminemia, increased sedimentation rate and C-reactive protein (CRP), hypocalcaemia and slightly increased levels of alkaline phosphatase (ALP) and gamma-glutamyl transferase (GT), but normal aspartate transaminase (AST) and alanine transaminase (ALT) levels. The patient quickly recovered from all symptoms after fluconazole withdrawal (Fig. 1a).

In 2008, she was admitted to the Rheumatology unit with frequent diarrhoea, systemic inflammation, cough and fatigue. On this occasion she was treated with azathioprine, prednisolone and phenytoin (600 mg daily) together with several other drugs (alfacalcidol, ampicillin, amoxicillin, calcium carbonate, clonidine, citalopram, furosemide, clonazepam, zopiclone, potassium, loperamide, methadone, gabapentin, pantoprazole, metoclopramide and propiomazine). Chest X-ray at admittance showed bilateral interstitial infiltrates, and she underwent a bronchoscopy where laboratory testing of broncho-alveolar lavage fluid revealed candidiasis. Therapy with fluconazole 400 mg a day was initiated. The incident from 2000 was not documented as an ADR in the computerized patient chart and was not known to the prescribers in 2008. After 2–3 days on fluconazole therapy, the patient developed ataxia, difficulties in walking, speaking and understanding what was said to her, increased tiredness and, ultimately, impaired consciousness. Laboratory results showed slightly increased CRP, anaemia, slightly increased ALP and gamma-GT levels, but normal AST and ALT levels. Several alternative diagnoses were discussed, such as endogenous depression, drug reaction to anti-anxiety agents and cerebral vasculitis. However, her husband reminded her that the symptoms resembled those of the phenytoin intoxication in 2000. The phenytoin P/S concentration, 14 days after start of fluconazole, once again was at an exceptionally high level, 240 μmol/L. Her cerebrospinal fluid (CSF) phenytoin concentration was 26.4 μmol/L, which reflects a normal plasma protein binding of about 90%. Following interruption of treatment with both fluconazole and phenytoin, she experienced a rapid decline in clinical symptoms of intoxication as well as P/S concentrations of phenytoin (Fig. 1b). A phenotyping test for CYP2C9 by losartan was performed [11, 12].

Methods

Phenytoin concentrations in the P/S and CSF reported here were determined by routine methods in the department of Clinical Pharmacology, Karolinska University Hospital, Stockholm, Sweden. The high-performance liquid chromatography (HPLC) analysis of phenytoin was performed after plasma protein precipitation by acetonitrile (67%) on an Agilent 1100 system (Agilent Technologies, Santa Clara, CA) equipped with a diode array detector monitoring using five wavelengths with 210 nm as the quantifying wave-

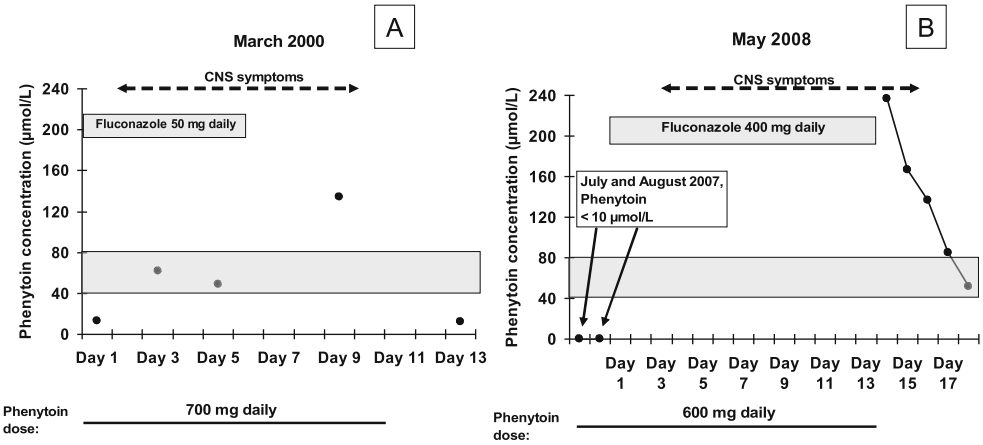

Fig. 1 **a**, **b** Schematic documentation of the two phenytoin–fluconazole interaction episodes. In March 2000, the patient showed central nervous system (*CNS*) symptoms a few days after the initiation of fluconazole treatment, despite phenytoin concentrations within therapeutic range (40–80 µmol/L, *shaded area*). The CNS symptoms were probably caused by the rapid increase in phenytoin concentrations

length. The analytical column was an Ace 3 C18 (3 µm, 50×3.0 mm; Advanced Chromatography Technologies, Aberdeen, Scotland). The mobile phase consisted of 10 mM natrium phosphate buffer, methanol and acetonitrile in the ratio of 60:30:10 (v/v), with buffer pH adjusted to 6.8 using 1 M potassium hydroxide solution. Chromatography was performed at 50°C at a flow rate of 0.3 mL/min. The method was linear between 10 and 400 µmol/L. The total precision (CV) of the method determined at 40 and 80 µmol/L was 3.0 and 2.5%. The accuracy estimated by participation in an international proficiency test programme was close to 97%.

The losartan test was performed after an overnight fast and after voiding the night urine. Losartan (Cozaar; Merck Sharp Dohme, Whitehouse Station, NJ) 25 mg was administered as a single oral dose in the morning, and urine was collected at 8 h thereafter. The urine sample was stored at −20 C until the HPLC analysis of losartan was performed. The metabolic ratio (MR) of losartan was determined by dividing the molar concentrations of losartan by that of its metabolite E-3174 [11]. The test was performed 6 months after the last phenytoin intoxication episode, and the patient had been off phenytoin treatment since that time. The *CYP2C9**2 and *3 and *CYP2C19**2 alleles were analyzed by TaqMan discrimination analysis (Applied Biosystems, Foster City, CA). The specificity was confirmed against that of Yasar et al. [12] and de Morais et al. [13]. Potential and relevant drug interactions were investigated by using the drug interaction database SFINX [14]. Finally, we searched a web-based database to determine whether any of the other drugs the patient was prescribed were CYP2C9 substrates, inhibitors or inducers [15].

Results

The concentration of losartan and its metabolite E-3174 in the 8 h-urine sample collected after a single oral dose of losartan was below the level of quantification (20 nM) and 149 nM, respectively. Thus, the MR was <0.13, revealing that the patient had a lower MR than any of the 190 healthy Swedish Caucasians used for comparison (Fig. 2) (Ramsjö et al. unpublished). This result confirms that this index patient is an outlier with ultra-high activity of CYP2C9. None of the *CYP2C9**2 and *3 or *CYP2C19**2 alleles were present in this patient, and she thus had the genotypes *CYP2C9**1/*1 and *CYP2C19**1/*1. Among the drugs the patient used that could be closely related to the two intoxication episodes, phenytoin was the only known substrate and fluconazole the only known inhibitor of CYP2C9. No other clinically relevant drug interactions could be found.

Discussion

The UM of drugs may have a genetic molecular origin, as demonstrated for substrates of CYP2D6 [1, 2, 5] and CYP2C19 [6, 16]. The induction of drug metabolism by drugs, such as rifampicin [17] and antiepileptic drugs [18], has been well documented. Phenytoin is a potent inducer

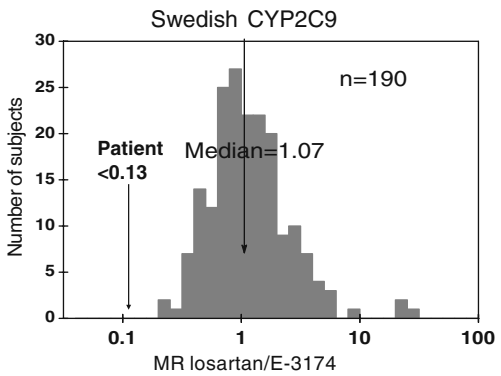

Fig. 2 The index patient had a metabolic ratio (MR) (losartan/E-3174) of <0.13, which is less than the MR in any of the 190 healthy Swedish Caucasians subjects used for comparison. The patient is thus an ultra-high metabolizer (UM) of the CYP2C9 substrate losartan

of CYP3A4, and since it induces its own metabolism it is probably also an inducer of CYP2C9, which recently has been shown in in vitro studies of human hepatocytes [19]. A strong autoinduction of phenytoin metabolism may be the reason for the UM in this index patient. However, the UM of the CYP2C9 probe drug losartan during a phenytoin-free period shows clearly that our patient has an inherent ultra-high activity of CYP2C9 not due to phenytoin autoinduction. We were able to exclude clinically relevant interactions, such as those if other drugs the patient used were a substrate, inhibitor or inducer of CYP2C9. Problems with the phenytoin assay due to interferences with co-medication could also be excluded using a diode array detector with five different wavelengths. Moreover, the samples were also analysed later on by a CEDIA immunoassay (Phenytoin II assay; Thermo Scientific, Waltham, MA) on an Olympus 400 with similar results.

There is a possibility that BD, a systemic vasculitis of unclear aetiology [20], may be related to the ultra-high activity of CYP2C9. Although we consider this possibility to be unlikely, it needs to be further studied. BD is an inflammatory disease, and inflammation may cause the downregulation—not induction—of drug metabolism [21]. Although found worldwide, BD is most prevalent along the ancient Silk Road, and the highest prevalence is found in Turkey. It is a disorder characterized by recurrent oral and genital ulcerations, skin lesions (papulopostural lesions and erythema nodosum) and chronic relapsing uveitis. In addition, BD may present with several different organ manifestations, including vascular, gastrointestinal and neurological manifestations. There is no specific diagnostic test for the disease [20].

A review of our patient's P/S phenytoin concentrations over the years revealed that the levels were below therapeutic range on several occasions. Poor compliance had been an issue, even though the patient denied this. In addition, NSAIDs had no effect. An uptake defect due to inflammation in the intestines was a differential diagnosis. Indeed, Chaleby et al. [22] reported a patient with BD showing a lack of response when treated with amitriptyline, diazepam, carbamazepine, phenytoin and acetaminophen. They performed a pharmacokinetic study and found subtherapeutic concentrations of amitriptyline and nonmeasurable concentrations of the other four drugs, which had been given at standard doses. Based on these results, the authors suggested that decreased drug absorption due to inflammatory changes associated with BD may have caused the subtherapeutic concentrations. The impact of the CYP enzymes was not studied, but the lack of measurable concentrations of phenytoin was similar to that in our patient. The result of the losartan test in our patient, namely, low urinary excretion of the parent drug and high concentrations of the metabolite, shows that the patient does not have a poor absorption of phenytoin—but that she has a high CYP2C9 activity.

In a study from Turkey, Tursen et al. [23] investigated *CYP2C9**2 and *3 in 62 patients with BD as compared to 107 healthy controls and found no difference in the frequency of these alleles. No phenotype of CYP2C9, as our test with losartan, was performed and, therefore, the CYP2C9 activity in these 62 patients is unknown.

The investigation of the patient´s chart also showed that she had suffered from suspected phenytoin intoxications on at least three other occasions, once when the patient was co-treated with nifedipine, once with metronidazole and once with fluoxetine, all known inhibitors of CYP2C9 [24–26]. The highest phenytoin P/S concentrations measured on these occasions was 96 μmol/L. However, the intoxication symptoms during these three episodes were not as severe as during the two episodes with fluconazole co-treatment as described above.

Conclusion

We present the first case of a patient with ultra-high activity of the cytochrome P 450 enzyme CYP2C9, similar to the well-studied ultra-high activity of CYP2D6 present in 1–10% of Caucasian populations. Our finding may have important clinical implications and explain some of the variability in the metabolic capacity reported for CYP2C9 substrates. In clinical practice, patients with ultra-high activity of CYP2C9 may need high doses of CYP2C9 substrates to reach therapeutic effect. Warfarin-treated patients may need high doses of warfarin to reach therapeutic international normalized ratio levels, and diabetic patients treated with oral antidiabetics may exhibit therapeutic failure when treated with ordinary doses of such drugs. Patients with UM of CYP2C9 may also metabolize the parent drugs to potentially hazardous active metabolites. The other important finding is that this case also clearly demonstrates the risk of severe intoxications in patients with UM when treated with a strong cytochrome P450 inhibitor, such as fluconazole. Warfarin-treated patients may be at risk for fatal bleedings when co-treated with inhibitors of this enzyme, and diabetic patients treated with oral antidiabetics may be at risk for severe hypoglycaemia. The molecular basis of our finding is now under investigation.

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Conflict of interest statement The authors declare that they have no conflict of interest.

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