The impact of *Cytochrome P450 2D6* and *UDP-glucuronosyltransferase 1A1* genotypes on the toxicity of antidepressants and tranquilizers

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Abstract

The cytochrom P450 (CYP) 2D6 enzyme is involved in the oxidative metabolism of many different classes of commonly used drugs, including antidepressants. The enzyme is highly genetically polymorphic, which results in metabolic capacity ranges between individuals from extremely slow, the poor metabolizer (PM), to extensive metabolizer (EM), or to ultrafast, the ultrarapid metabolizer (UM). CYP 2D6 deficiency can be a risk factor for adverse drug effects and increased toxicity in poor metabolizers when such patients are treated with standard doses of anti-depressants. We report two cases of poisonings with the antidepressants amitriptyline and trimipramine in different *CYP 2D6* genotypes. For the trimipramine poisoning in a *CYP2D6*4/*5* genotye PM the calculated half-life was 3-5 fold higher compared to a poisoning in EM with amitriptyline. In both cases the plasma concentrations of tricyclic antidepressants were in the lethal ranges.

The uridine diphosphate (UDP)-glucuronosyltransferases (UGTs) are key enzymes in human phase detoxification of drugs, which are generally transformed into water-soluble glucuronides. Gilbert syndrom is characterized by a mild hyperbilirubinemia and caused by an insertional mutation of a TATAA element in the *UGT1A1* gene causing a reduced enzyme activity. In a young girl poisoned with diazepam and suffering of Gilbert syndrome we observed markedly prolonged half-lives for the parent compound and the metabolites, especially oxazepam. If the changed pharmacokinetic data are in the context of this gene defect remains unclear.

1. Introduction

Great heterogenity exists in the way individuals respond to drug therapy. Reasons for this variability include pathophysiological or environmental factors, drug interactions and individual variation. Genetic factors contribute to the phenotype of drug reponses. Xenobiotic metabolizing enzymes play central roles in biotransformation and detoxification but may contribute also to toxification of compounds. Hence, therapy with psychotropic drugs is characterized by high inter-individual variability in effect size and inherited polymorphisms can lead to altered drug response [1;2].

Among those influences are polymorphisms in drug-metabolizing phase I enzymes such as the members of the cytochrome P450 superfamily CYP2D6, 2C19, and 2C9 [3;4]. The situation becomes more complicated, as these enzymes cause inter-individual differences in drug metabolism ranging from extremely fast metabolism to no metabolism at all. As a result this has consequences for the effi-

cacy or toxicity of antipsychotic drugs [5]. About 5 % of Caucasians are ultrarapid metabolizer (UM), and, on the contrary, about 7 to 10 % are poor metabolizer (PM), inducing very different rates in the transformation of antidepressants metabolized by CYP 2D6. The lack of activity in PM is caused by two deficient alleles in the *CYP 2D6* gene, while a gene duplication results in high enzyme activity. Subjects with two active alleles are classified as extensive metabolizer (EM), and carriers of one active and one deficient allele are determined as intermediate metabolizers (IM). This group is assumed to have subpopulation-specific clearance between EMs and PMs [6]. The clinical importance of these variants depends on the allele-frequency, on the therapeutic range of the drug and on duration of administration. Tricyclic antidepressants such as amitriptyline [7], nortriptyline [8], trimipramine [9], and numerous further drugs [10] metabolized via CYP 2D6 show huge differences in plasma concentrations as well as in pharmacokinetic parameters and dose adjustments are necessary for an individual treatment [11].

UDP-glucuronosyltransferase (UGT) enzymes catalyze the conjugation of various endogenous substances, e.g. bilirubin, steroids and biliary acids, and exogenous compounds as drugs, environmental agents or carcinogens. Glucuronidation is one of the major phase II drug metabolizing reactions generating water-soluble products for excretion in bile or urine and, in most cases, glucuronidation usually abolishes the pharmacological activity. The UGTs are encoded by a multigene family in humans and genetic variations lead to numerous polymorphisms. The human UGT superfamily is comprised of two families, UGT1 and UGT2, and three subfamilies, UGT1A, UGT2A, and 2B [12-14]. The *UGT1A* gene encodes 9 functional proteins. UGT1A1 is a major conjugating enzyme that is responsible for the homeostasis of bilirubin and the glucuronidation of a wide selection of xenobiotics [15]. The multiplicity of transferases show overlapping substrate specifity and may provide functional compensation for genetic deficits [16-18].

Gilbert syndrome and the Crigler-Najjar syndromes are disorders of bilirubin conjugation with consecutive indirect hyperbilirubinemia of different severity. Morbus Gilbert is a mild hyperbilirubinemia, which is only of significance in case of drug therapy. A common genetic polymorphism in the promoter region of the *UGT1A1* gene, denoted as *UGT1A1*28*, leads to reduced enzyme expression [19]. This genotype causes reduced elimination of some drugs as ethinylestradiol [20], lorazepam [21], or irinotecan [22]. Nevertheless, plasma clearance of most drugs that undergo glucuronidation is unaffected and the risk for adverse clinical effects remains theoretical.

We present two case reports on a 27-year old woman with normal 2D6 metabolic capacity poisoned with amitriptyline in suicidal attemption and a second case on a 34-year old woman treated with trimipramine, who was classified as poor metabolizer. In a third case in a patient who has poisoned with diazepam and suffered at Gilbert syndrome we observed markedly prolonged half-lives for the parent compound and the metabolites.

2. Case histories

2.1 Antidepressant poisonings

A 27-year old woman poisoned with an unknown amount of amitriptyline in suicidal attention. In a second case, a 34-year old woman, who was treated with trimipramine, took an overdosage. On admission, both patients were in deep coma, accompanied by hypotension, arrhythmia, seizures, and anticholinergic symptoms. They received gastric lavage, active charcoal and were treated symptomatically. Toxicological screening revealed the corresponding antidepressants in blood and urine.

2.2 Diazepam poisoning

A 16-year old girl had taken an overdose of diazepam. She was admitted to the Intensive Care Unit (ICU) with drowsiness and a marked muscular weakness. Long lasting sleep was a further clinical symptom. Diazepam and metabolites were determined in serum and urine after acid hydrolysis by gaschromatography-mass spectrometry (GC-MS). Gilbert syndrome was diagnosed earlier. No therapy was necessary.

3. Procedures

3.1 Chemicals

All chemical used were of analytical reagent standard. Amitriptyline, trimipramine, diazepam, nordiazepam, and oxazepam were purchased from Sigma-Aldrich (Taufkirchen, Germany), and the deuterated internal standards were obtained by Promochem (Wesel, Germany).

3.2 Patient samples and sample preparation

Preparation: Blank plasma samples used for calibration curves were obtained from the local blood bank. Patient samples were submitted to the laboratory by the hospitals for toxicological analysis. The tricyclic antidepressant probes were processed after addition of amitriptyline-d^3 and trimipramine-d^3, respectively, as described previously [23]. The tranquilizer probes were analysed as described in [24] with diazepam-d^5 as internal standard.

Genotyping: DNA was extracted from the blood sample using a standard phenol-chloroform extraction method. Analyses for the *CYP2D6* alleles were performed with polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods as described previously [25].

3.3 Instrumentation

Gaschromatography-mass spectrometry (GC-MS) analysis: The samples were analyzed in the EI-SIM method using an Agilent (Agilent, Waldbronn, Ger-

many) 6890 gaschromatograph combined with an HP 5973 mass spectrometer. The GC was equipped with an HP 5MS capillary column.

Pharmacokinetics: The PC Origin Programm (Microcal 5.0, Soft Guide, Wolfsburg, Germany) was used for the modelling of the plasma concentration data.

4. Results

4.1 Antidepressant poisonings

The quantification of amitriptyline and trimipramine showed toxic values in the sera of both patients. While the terminal half-life of amitriptyline elimination is with $t_{1/2}$= 46.4 hours in the range for this compound, the half-life in the patient intoxicated with trimipramine is with $t_{1/2}$= 162.0 hours markedly prolonged. Genotyping of the blood sample yielded two nonfunctional *CYP2D6* alleles without CYP2D6 activity. A poor metabolizer genotype *4/*5 with a splicing defect in the *4 allele and a complete gene deletion in the *5 allele was detected. Both concentration time curves were charaterized by a rapid decline of the concentration and a slow elimination. The plasma pharmacokinetics of drug elimination was fitted by a two-compartment modell, Fig. 1 and 2.

4.2 Diazepam poisoning

The concentration time course after diazepam poisoning in a girl with Gilbert syndrome was charaterized by markedly prologed half-lives for the parent drug and the metabolites, especially for oxazepam. Figure 3 shows a scheme for the biotransformation and excretion of diazepam and the major active metabolites nordazepam and oxazepam with the half-lives calculated.
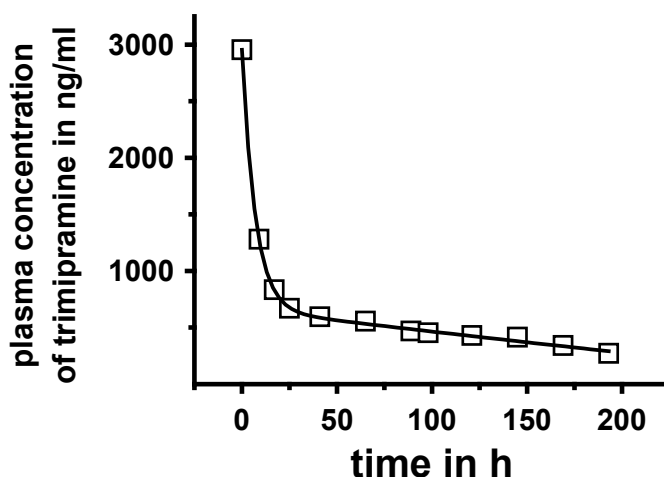
Figure 1. Decline of plasma concentration after amitriptyline poisoning in an extensive metabolizer fitted by a two-compartment modell and a first order kinetic. The curve represents a quick distribution with a half-life of $t_{1/2\alpha}$= 2.9 hours and a prolonged elimination phase with $t_{1/2\beta}$= 46.4 hours. The therapeutic plasma concentrations are between 80-300 ng/ml.
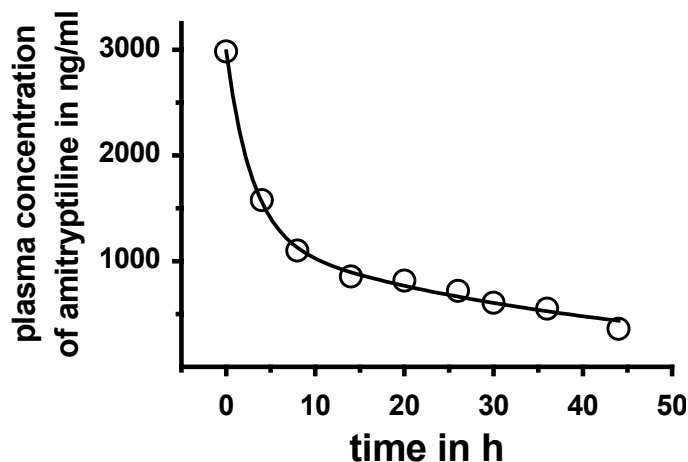
Figure 2. Decline of plasma concentrations in a trimipramine poisoning of a poor metabolizer fitted by a two-compartment modell and a first order kinetic. The patient had a *CYP2D6*4/*5* genotype without functional enzyme activity. The curve is characterised by a biphasic elimination with a distribution half-life of $t_{1/2\alpha}$= 4.7 hours and a terminal half-life $t_{1/2\beta}$= 162.0 hours. Therapeutic plasma concentrations are between 10-250 ng/ml.
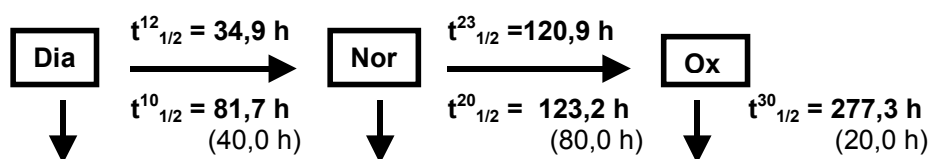

Figure 3. Kinetic model of diazepam biotransformation and excretion of the parent substance and the metabolites with half-lives calculated. The half-lives from normal subjects are shown in parenthesis.

The measured plasma concentrations are shown in Figure 4. The concentration time courses were modelled by a differential equation system. The half-life for oxazepam is about 13 times higher compared with normal cases. The excretion was complete after two weeks.

5. Discussion

5.1 Antidepressant poisonings

Most tricyclic antidepressants are handled similarly to amitriptyline and nortriptyline, however, the pharmacology of amitriptyline is complex. Metabolism of amitriptyline results in potentially eight active moieties including the hydroxy metabolites of each moiety. CYP 2D6 mediates the conversions to hydroxy-amitriptyline and hydroxynortriptyline, whereas demethylation of amitriptyline to nortriptyline is caused by multiple CYPs, especially CYP2C19. Subsequent meta-
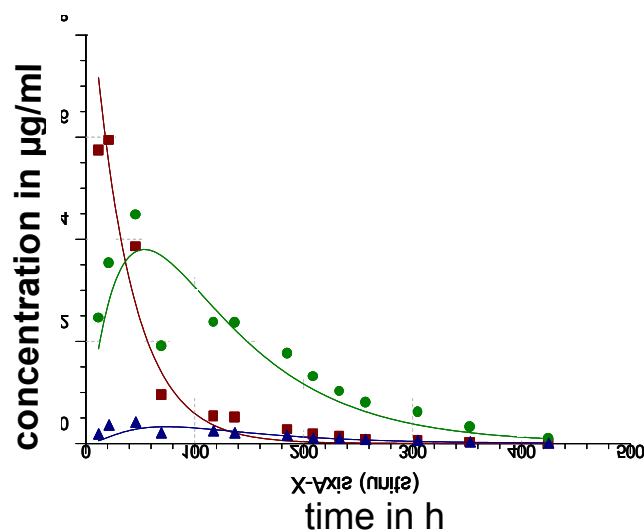
Figure 4. Plasma concentrations and pharmacokinetic modelling of diazepam (■), nordiazepam (●), and oxazepam (▲) in a patient with Gilbert syndrom poisoned with diazepam. The excretion for diazepam and metabolites is extremly prolonged compared with normal half-lives (see Fig.3).

bolism of hydroxyderivatives is generally via glucuronidation. In extensive metabolizers, as in our case, elimination half-lives were in the range between 30-50 hours [26]. Intermediate metabolizers with one dysfunctional *CYP2D6* allele have a greater risk of side effects than those with two functional alleles, and this risk was associated with higher nortriptyline concentrations [8;27].

Trimipramine is biotransformed to the main metabolites desmethyl-trimipramine, di-desmethyl-trimipramine, 2-hydroxy-trimipramine, and 2-hydroxy-desmethyl-trimipramine. Pharmacokinetics of the parent substance and its demethylated metabolites strongly depend on the *CYP2D6* genotype. The bio-availability of trimipramine is at least 3–fold higher in PMs than in EMs [9]. In patients with normal metabolic capacity half-lives have been found to range from 16-39 hours, while in our case with the two *4/*5 deficient alleles the half-life is 3-5 times higher. Further *CYP2C9* as well as *CYP2C19* are, to a lesser extent, involved in N-demethylation of trimipramine and other tricyclic antidepressants.

Overdoses with tricylic antidepressants are serious situations since the manifestations of poisoning are severe and difficult to control. Optimal patient response appears to be achieved if plasma concentrations lie between 80-200 ng/ml for amitriptyline and 10-250 ng/ml for trimipramine. In several fatalities, concentrations of amitriptyline or trimipramine range from 1 500-15 000 ng/ml [26]. However, myocardium concentrations are approximately 5 times the respective blood concentrations. This may explain the discrepancy between plasma concentrations and the severity of toxic symptoms since blood concentrations are not guidelines for the treatment in antidepressant poisonings [28].

5.2 Diazepam poisoning

The half-lives of diazepam, nordiazepam, and oxazepam were estimated as 21-37 hours, 50-99 hours, and 6-20 hours, respectively, in normal volunteers [26]. While in our patient with Gilbert syndrome the half-life especially for oxazepam is extremly prolonged, we assume a correlation between the glucuronidation defect in *UGT1A1* gene and the excretion, but we have no evidence. UGT1A1 is not considered to be a key enzyme in the diazepam or oxazepam glucuronidation. Relatively little has been reported concerning the glucuronidation of diazepam and metabolites. Oxazepam is one of the C-3-hydroxylated benzodiazepines for which glucuronidation is the predominant pathway [29;30]. S-oxazepam was shown to be polymorphically glucuronidated by UGT2B15, while R-oxazepam is glucuronidated by UGT2B7 and UGT1A9 isoforms [31]. Patients affected by Gilbert syndrom display lower glucuronidation for e.g. lorazepam [32]. In phase I CYP2C19 and 3A4 mediate 33 and 44 % of the biotransformation of diazepam to nordiazepam and also play a role in producing of temazepam and oxazepam [33;34].

6. Conclusion

Tricyclic antidepressant and, in particular, amitriptyline and trimipramine are cornerstones in the therapy of psychiatric disorders. Adverse effects may often be linked to polymorphisms of drug metabolizing enzymes and the identification of genotypes is more important than currently anticipated. Inter-individual variations in patient response is caused by genetic polymorphisms of cytochrom P450 2D6. Knowing the patients genotype, it may be possible to predict the drug dosage for that patient, thus improving therapeutic response and avoiding toxic events. In patients with CYP2D6 deficient metabolic capacity the dosage will have to be reduced below the standard dosage.

Gilbert syndrome is associated with *UGT1A1*28* (TA$_7$) polymorphism and it is likely that patients with this allele will present an altered drug clearance compared to patients with the wild type gene. However, for most benzodiazepines an altered rate of glucuronidation do not exist and systematical trials are missing.

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8. References

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