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Pharmacogenetic aspects of tramadol pharmacokinetics and pharmacodynamics after a single oral dose

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ABSTRACT

The major purpose of this study was to elucidate if genotyping can facilitate interpretations of tramadol (TRA) in forensic case work, with special regard to the estimation of the time of drug intake and drug related symptoms (DRS). The association between genetic polymorphisms in *CYP2D6*, *OPRM1* and *ABCB1* and pharmacokinetic and pharmacodynamic properties of TRA was studied. Nineteen healthy volunteers were randomized into two groups receiving a single dose of either 50 or 100 mg of orally administrated TRA. Blood samples were collected prior to dosing and up to 72 h after drug intake. The subjects were asked to report DRS during the experimental day. We found a positive correlation between the metabolic ratio of *O*-desmethyltramadol (ODT) to TRA and the time after drug intake for both CYP2D6 intermediate metabolizers and extensive metabolizers. For the only poor metabolizer with detectable ODT levels the metabolic ratio was almost constant. Significant associations were found between the area under the concentration-time curve (AUC) and three of the investigated *ABCB1* single nucleotide polymorphisms for TRA, but not for ODT and only in the 50 mg dosage group. There was great interindividual variation in DRS, some subjects exhibited no symptoms at all whereas one subject both fainted and vomited after a single therapeutic dose. However, no associations could be found between DRS and investigated polymorphisms. We conclude that the metabolic ratio of ODT/TRA may be used for estimation of the time of drug intake, but only when the *CYP2D6* genotype is known and taken into consideration. The influence of genetic polymorphisms in *ABCB1* and *OPRM1* requires further study.

1. Introduction

The use of tramadol (TRA) for treatment of moderate to severe pain has been increasing steadily during the last decades. Unfortunately, abuse of this drug is also becoming more common. Further, development of addiction to TRA in association with analgesic treatment within the recommended dose range is another alarming trend. A history of abuse or use of a drug of abuse seems to be an important risk factor [1]. TRA has also become a more common cause of death in drug addicts with a similar trend for increase in overdose cases [2,3].

Analysis of drugs of abuse is a common feature of forensic investigations and correct interpretation of the measured concentrations is important in both post mortem and human performance toxicology. Accurate estimation of the time of drug intake and expected drug effects from a certain dose or concentration are also frequent issues in drug-facilitated crimes.

TRA has a dual mechanism of action, acting as a μ-opioid receptor agonist as well as a serotonin and norepinephrine reuptake inhibitor. The cytochrome P450 (CYP) enzyme CYP2D6 is involved in the formation of the active metabolite *O*-desmethyltramadol (ODT). In comparison to TRA, ODT is a significantly more potent μ-opioid agonist [4]. The concentration of the parent compound alone is often not sufficient to make an accurate estimation of the time of drug intake. The ratio between metabolite and parent compound is generally used to indicate a recent acute intake, e.g. to diagnose suspected acute overdoses. It has earlier been shown, for some substances other than TRA, that the ratio between metabolite and

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parent compound also can be helpful in a more accurate estimation of the time of intake [5,6]. The amount of ODT formed is largely dependent on the *CYP2D6* genotype but also on the time of intake. It is however unclear if the metabolic ratio (MR) of ODT/TRA is a useful indicator of the time of TRA intake.

There is considerable variation in the interindividual response to the same dose of opioids, including TRA, with respect to both therapeutic and adverse effects. Development of tolerance due to prolonged use of opioids in addition to genetic factors could partly explain some of these differences. Several genes and polymorphisms have been studied in this respect. Reduced or absent metabolite formation with reduced analgesic effect have been observed after TRA administration in CYP2D6 poor metabolizers (PMs) [7], whereas ultrarapid metabolizers (UMs), are instead associated with increased analgesic effects, but with higher risk for adverse effects [8].

The *ABCB1* gene encodes P-glycoprotein (P-gp), which is located in the blood–brain barrier and gut and is responsible for the cellular efflux of a variety of drugs [9]. The most common single nucleotide polymorphisms (SNPs) in the coding region are C1236T, G2677T and C3435T [10]. C3435T, the most studied SNP, has been associated with both increased and decreased expression of P-gp [9]. An altered expression in gut could potentially change the pharmacokinetics of the drugs being substrates of this transporter. A decreased expression and functionality of P-gp has been suggested in homozygous for the 3435T allele [11], possibly leading to higher bioavailability and subsequently a higher concentration of TRA in blood. Although P-gp is of importance for the pharmacokinetic and pharmacodynamic properties of a number of substances, including opioids [9,12], its importance for TRA remains unknown. A clinical study suggested that TRA is a substrate of P-gp [13]. In contrast, an in vitro study and one in vivo study in rat have demonstrated that TRA and ODT are not P-gp substrates [14,15]. A recent study showed no association between the C3435T polymorphism and pain relief in patients receiving TRA [16]. Taken together, the importance of P-gp for the pharmacokinetic and pharmacodynamic properties of TRA still remains to be corroborated.

Genetic polymorphisms in *OPRM1* have been associated with an altered pain threshold and opioid requirements. Results from clinical studies have shown that patients homozygous for the *wild-type* of the most common SNP A118G require less opioids than those with the other allelic variants, AG and GG [17,18]. It has also been shown that the 118G allele is associated with a more severe clinical outcome in emergency department patients with acute drug overdose [19]. There is only limited information regarding the influence of *OPRM1* A118G polymorphism on TRA efficacy [20].

We undertook a human study to elucidate if genotyping can facilitate interpretations of TRA in forensic case work, with special regard to the estimation of the time of drug intake and drug related symptoms (DRS). Our study had the following specific aims:

(1). Investigate if the metabolic ratio (MR) of ODT/TRA is a useful indicator of the time of TRA intake.
(2). Determine the association between polymorphisms in the *ABCB1* gene (SNPs G1199A, C1236T, G2677T/A, C3435T) and pharmacokinetic parameters of TRA.
(3). Study the association between polymorphisms in the *CYP2D6*, *OPRM1* (SNP A118G) or *ABCB1* (SNPs G1199A, C1236T, G2677T/A, C3435T) gene and DRS.

2. Methods

2.1. Study participants and study design

Twenty healthy volunteers were recruited through advertisements. Nineteen subjects (nine males, ten females) aged 18 years or older (mean 25.4 ± 4.3) completed the study, whereas one subject did not show up on the experimental day due to unspecified illness. Participants were informed and examined by a physician before inclusion in the study. Questions of present or previous drug use were asked. Previous or concurrent use of opioids or drugs known to interact with TRA were exclusion criteria. Demographic data such as age, gender, height and weight were noted. Seven of the female participants were taking oral contraceptives. None of the participants stated upon question that they were pregnant or breast-feeding. Written informed consent was collected from each participant. The study was approved by the Regional Ethical Review Board in Linköping (No: 2011/337-31). The subjects were randomized into two groups receiving a single dose of either 50 or 100 mg of orally administered TRA (Tramadol HEXAL, Sandoz). During the experimental day, blood samples were obtained from a peripheral venous catheter, Insyte W in combination with a Mandrin (Becton Dicinson AB), which was inserted in the forearm. The blood was collected in labeled 7 ml Na-heparinized collection tubes using a Vacutainer Luer-Lok Access Device (Becton Dicinson AB). Blood samples were collected prior to dosing and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 24, 48 and 72 h. Samples were stored at −80 °C pending analysis. The participants were exhorted to eat breakfast according to their usual routines. Lunch was bought from a nearby restaurant and served around noon. Fruit, some biscuits, tea, coffee, juice and water were available during the whole day. The subjects were requested to fill in a form regarding their experience of DRS during the experimental day in conjunction to the last blood sampling on the first day (10 h). Seven questions about nausea, dizziness, headache, vomiting, dry mouth, sweating and fatigue were posed. A scale between zero to five were used, where zero was no symptoms at all and five was worst imaginable symptoms.

2.2. Quantitation of tramadol and O-desmethyltramadol in whole blood

2.2.1. Chemicals and reagents

Acetonitrile (gradient grade), methanol (gradient grade) and formic acid (98%) were purchased from Merck (Darmstadt, Germany). Ammonium formate (98%) and ethanol (95%) were purchased from Fluka (Basel, Switzerland) and Kemetyl (Haninge, Sweden), respectively. Water used was first purified with a MilliQ-water purifying system (Millipore Corporation, Bedford, MA, USA). Reference substances used for making calibrators and quality controls (QCs), i.e. *cis*-tramadol and *O*-desmethyl-*cis*-tramadol were purchased from Cerilliant (Austin, TX, USA). Tramadol-^{13}C-D_3 and *O*-desmethyl-*cis*-tramadol-D_6, used for making the internal standard were also purchased from Cerilliant.

2.2.2. Instrumentation

High performance liquid chromatography was performed on a 1290 Infinity LC instrument (Agilent Technologies), using a 2.1 × 100 mm Zorbax Eclipse Plus C18 RRHD column with 1.8 μm particles. A guard filter with 0.2 μm particles (Waters) was also used. Column temperature was set to 60 °C. Mass detection (MS/MS) was performed on an AT6460 instrument (Agilent Technologies) with an electrospray interface, using positive ionization. Mobile phase A consisted of 0.05% formic acid in 10 mM ammonium formate while mobile phase B consisted of 0.05% formic acid in methanol. Gradient elution with a total run time of 8 min was used. The total flow rate was 0.5 ml/min. The software used was Masshunter (Agilent Technologies). Identification criteria were based on transition ratios. Both TRA and ODT exhibit a very strong base peak of 58.1 and few other fragments, which have abundance less than 10% of base peak. In accordance with European recommendations (EUD 2002/657/EC) an acceptance limit of 50% was used. The following transitions were used