

Plasma levels of tramadol and O-desmethyltramadol enantiomers in patients with different CYP2D6 genotypes

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Abstract

Objective: The influence of the number of active CYP2D6 alleles on enantiomeric plasma levels of tramadol (T) and its M1 metabolite O-desmethyltramadol (ODT) and the response to postoperative tramadol analgesia was investigated.

Methods: After abdominal surgery the plasma levels of T and ODT enantiomers were measured after an intravenous bolus application of tramadol of 3 mg/kg body weight. Subsequent postoperative analgesia over 48 hours was performed by patient controlled analgesia. Blood samples were drawn 30, 90 and 180 min after initial infusion of T. Concentrations of the T enantiomers (+)-T, (-)-T, and metabolites (+)-ODT and (-)-ODT were analyzed by liquid chromatography–tandem mass spectrometry. Genotyping was performed for CYP2D6 polymorphisms and the gene duplication by PCR and real-time PCR.

Concentration of T and ODT enantiomers between patients with no, one, two, or at least three functionally active CYP2D6 alleles as well as response to T medication were compared.

Results: Demographic and surgery related data were comparable between the four different genotypes. Number of active alleles was correlated to metabolic capacity of CYP2D6. Patients with no active CYP2D6 allele showed the highest levels for T and negligible plasma levels for +ODT. Non response rates to pharmacologic treatment in patients with no active allele were more than doubled compared to patients with at least one wild type allele ($p < 0.001$).

Conclusions: CYP2D6 genetic variations determine plasma levels of T and ODT enantiomers and influence efficacy of T treatment.

1. Introduction

Polymorphisms within cytochrome P450 2D6 (CYP2D6) have been associated with altered enzyme activity. In contrast to carriers of two wild type alleles (extensive metabolizers = EMs), heterozygous individuals (HZ) with only one functionally active allele, intermediate metabolizers with two variant alleles known to decrease enzymatic capacity (IMs) and poor metabolizers (PMs) with no functionally active allele are predicted to have either reduced or no enzyme activity, respectively. PMs display a frequency of about 7-10% in Caucasian populations [1,2]. They are at increased risk to suffer from adverse drug effects by overdose from β -blockers, antiarrhythmics, tricyclic antidepressants, antipsychotics etc.. Blood levels might exceed the therapeutic range because of lacking activity of CYP2D6. In contrast, ultra rapid metabolizers (UMs) display a CYP2D6 gene duplication or multiduplication which significantly increased

enzyme activity [3,4]. This can result in therapeutic failure due to drug levels below the therapeutic range.

In case of pro-drugs like codeine and tramadol, PMs experience no or reduced analgesia, respectively [5,6]. Tramadol hydrochloride (T) is a weak opioid with its μ -opioid receptor analgesic properties preferentially mediated by its M1 metabolite *O*-desmethyltramadol (ODT). Furthermore, the clinically used racemic mixture of the trans-isomer inhibits noradrenaline and serotonin reuptake, which also contributes to its analgesic properties.

A study was conducted in a clinical setting, enrolling patients under concomitant medication who recovered from major abdominal surgery. It describes the course of sequential enantiomer concentrations of T and its major metabolite ODT, which mediates T efficacy within the most important 3 hours following T administration. Dependence of enantiomer concentrations on genotype uncovers further the impact of genetic variation on efficacy of this pain medication.

2. Clinical study protocol

After giving written informed consent, 187 patients (ASA classification I-III) scheduled for elective, larger abdominal surgery were instructed in the details of the study. General anesthesia for abdominal surgery and postoperative analgesia were conducted using a standardized protocol: propofol 2 mg/kg, fentanyl 0,2 mg and cis-atracurium for induction and remifentanyl, isoflurane 1 MAC and cis-atracurium for maintenance of anesthesia. About 90 minutes before termination of anesthesia an intravenous loading dose of tramadol (Tramal[®], Grunenthal GmbH, Aachen, Germany) 3 mg/kg (maximum loading dose 250 mg) and dipyrone 1 g i.v. was infused via infusion pump over 15 minutes. After emergence of anesthesia, about 90 minutes after the infusion of the study medication, patients were transferred to the recovery room where they were monitored for further 90 minutes. When patients had recovered from anesthesia they were asked for pain intensities. At pain scores >40 at rest on the NRS they received a further dose of T 50 mg, which could be repeated after 30 minutes. If analgesia remained insufficient in the post-anesthetic care unit, rescue medication piritramide in 2 mg increments was given i.v..

For further analgesic treatment on the general ward, a PCA device (Injektomat^R-CP PACOM, Fresenius AG, Bad Homburg, Germany) was provided and patients could self-administer intravenous bolus doses of tramadol 20 mg and dipyrone 200 mg. The delivery time of one bolus dose was 1.5 minutes and the lock-out time was 8 minutes.

In case of prolonged elevated pain scores additional rescue medication piritramide was administered, or analgesic regime was changed to piritramide via PCA.

3. Laboratory analyses

Blood samples were obtained with ethylenediaminetetraacetic acid (EDTA) as anticoagulant 30, 90 and 180 minutes after tramadol infusion. The whole blood was centrifuged and plasma and cells were separated and frozen at -80°C until analysis. Quantification of (+)-T and (-)-T as well as (+)-ODT and (-)-ODT was performed by a liquid chromatographic-mass spectrometric assay with atmospheric pressure chemical ionization (LC-APCI-MS) [7]. For genotyping, genomic DNA was isolated from whole blood using the Puregene DNA Isolation Kit (Gentra Systems, Inc., Minneapolis, USA). The further procedure was recently described in detail [8].

4. Results

The results of genotyping are described in Table 1. Complete chemical-toxicological results with enantiomeric T and ODT concentrations were available in 174 patients. Quantification of T enantiomers revealed high plasma levels, with both enantiomers ranging between 55-1100 ng/ml.

Tab. 1: Number and percent of patients allocated to the different genotypes.

	n	%	metabolism
EM	68	36.4	normal
HZ/IM	93	49.7	reduced
PM	18	9.6	no
UM	8	4.3	increased

T concentrations were highest 30 min after infusion and decreased during the following 2.5 hours with UM showing the most pronounced metabolism. ODT was found in much lower concentrations than the parent drug. Concentrations at 30, 90 and 180 min for all patients amounted to 22.1 ± 3.1 , 22.8 ± 2.0 and 30.3 ± 2.8 ng/ml for (+)-ODT and 51.9 ± 7.3 , 52.1 ± 4.8 and 57.0 ± 4.9 for (-)-ODT, respectively. Concentration of ODT showed an increase over 180 min in carriers with at least one active allele (Fig. 1a). 90 and 180 min after T administration ODT levels were significantly higher in these genotypes compared to PMs ($p<0.001$).

Variability of drug and metabolite concentrations was high. This was in part due to pretreatment with co-medication which inhibits cytochrome activity (e.g. long-term medication of antidepressants, metoclopramide, ranitidine or others). Figure 1b differentiates patients with at least one functional CYP2D6 gene in subgroups with or without co-medication. In patients with one and two active genes and long-term co-medication with CYP2D6 inhibitors concentrations

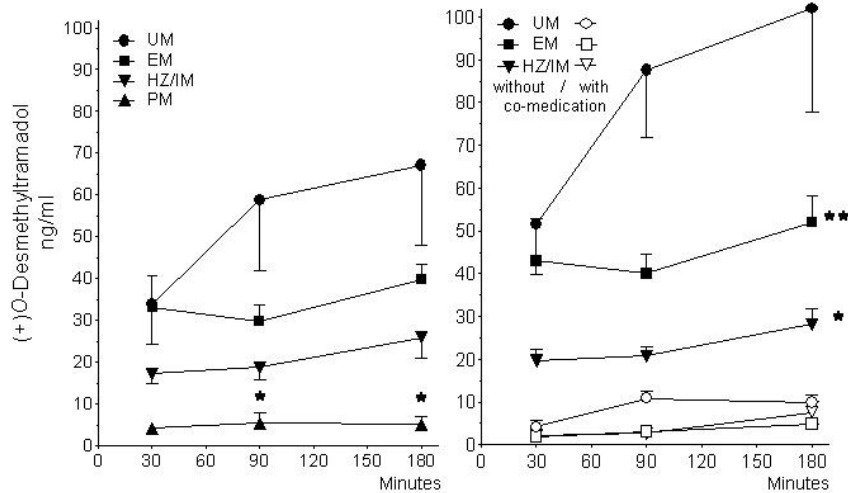


Fig. 1: Concentration of (+)-ODT (mean±SD) at 30, 90 and 180 min after intravenous bolus dose of tramadol. **a:** allocated to genotype groups UM (n=8), EM (n=62), HZ/IM (n=85) and PM (n=18), * p<0.001 at 90 and 180 minutes. **b:** allocated to genotype groups without and with co-medication: UM (6 patients without / 2 patients with co-medication), EM (47/15 patients) and HZ/IM (77/8 patients). * p<0.05 for group PM without versus PM with co-medication, ** p<0.001 for group EM without versus EM with co-medication.

for both ODT enantiomers were significantly lower compared to the respective genotype without co-medication. AUCs of T did not differ between the (+)- and (-)-enantiomer. Due to the high variability of the enantiomer concentrations, specifically in PMs and HZ/IM individuals there was no significant difference for concentrations between the genotypes. AUCs of (+)- and (-)-ODT, both clearly separated the PM subjects from the other genotypes with PMs displaying the lowest values (Kruskal-Wallis-test: p<0.001). There was no difference between HZ/IMs and EMs, as well as EMs and UMs. In general, concentrations of (-)-ODT were higher than of (+)-ODT.

177 patients completed a 48-hour PCA period and analgesic response could be evaluated. In the post-anesthesia care unit 86 patients (PM: 10, HZ: 24, EM: 18, UM: 6) had pain scores >40 and received a second dose of T 50-100 mg. Ten PMs needed rescue medication piritramide in the postoperative care unit, significantly more than patients with at least one wild-type allele (p<0.001) (Fig. 2).

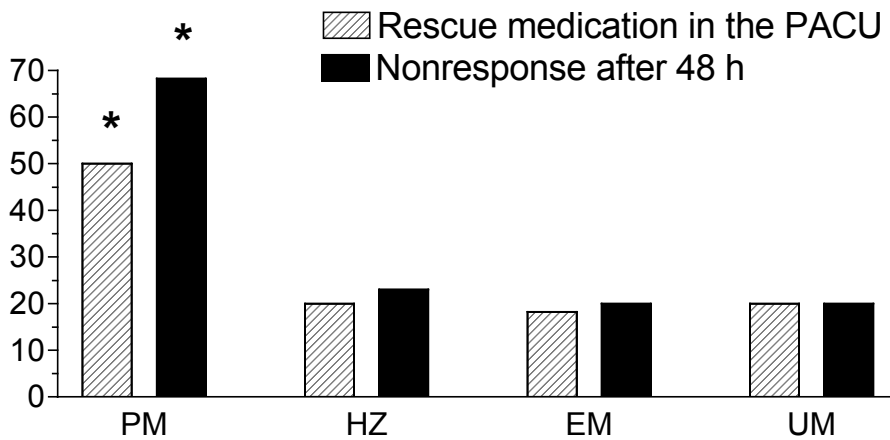


Fig. 2: Non responders to T treatment allocated to genotypes. White columns: % of patients needing rescue medication in the postanesthetic care unit (PACU); black columns: % of non responders after the 48 hour study period. * $p < 0.001$ for the PM group compared to the HZ/IM, EM and UM group.

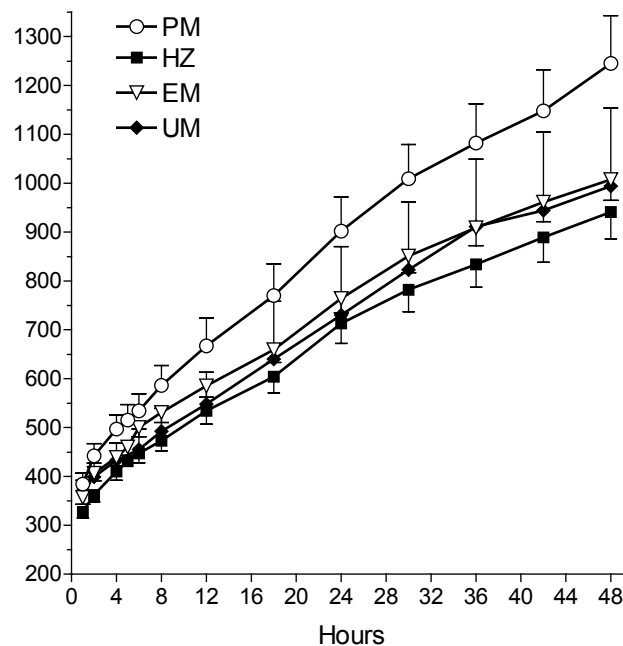


Fig. 3: Cumulative analgesic consumption (mean \pm SEM) over the 48 hour study period in the PM (n=16), HZ/IM (n=89), EM (n=64) and UM group (n=8). T loading dose, T bolus application via PCA and rescue medication piritramide (conversion piritramide : tramadol =1:10) are considered.

Cumulative analgesic consumption allocated to genotypes is displayed in Fig. 3. PMs needed more analgesics compared to HZ/IM subjects and EM subjects after 24 and 48 hours ($p=0.002$). Due to the small number of patients in the UM group, statistical analysis of the analgesic consumption in this group was not feasible. After the 48-hour study period more PMs (81.3 %) were categorized

as non responders compared to patients with CYP2D6 activity ($p < 0.001$). There was no difference in response rates between patients of the HZ/IM group, EMs and UMs.

5. Conclusions

Enantioselective analysis of T and ODT was performed in a clinical setting enrolling patients recovering from major abdominal surgery. Variability of ODT concentrations were closely correlated to CYP2D6 genotypes. Concomitantly used CYP2D6 inhibitors also contributed to variability of T metabolism. Non response rates to pain medication increased fourfold in PM and thus, this genotype was associated with poor efficacy of T analgesia. The whole study was recently published in detail [8].

6. References

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