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Authors' names: Karolína Hronová[1], Pavla Pokorná[1,2,3], Martin Šíma[1,2], Ondřej Slanař[1]

Affiliations:

[1] *Institute of Pharmacology, First Faculty of Medicine, Charles University, Albertov 4, 128 00, Prague 2*

[2] *Clinic of Pediatrics and Adolescent Medicine, General University Hospital, Ke Karlovu 2, 121 00, Prague 2*

[3] *Intensive Care and Department of Paediatric Surgery, Erasmus MC - Sophia Children's Hospital, Rotterdam, the Netherlands, Wytemaweg 80, 3015 CN Rotterdam, the Netherlands*

Address for correspondence:

Department of Pharmacology, First Faculty of Medicine, Charles University and General University Hospital in Prague, Albertov 4, 128 00 Prague 2, Czech Republic

E-mail: khron@lf.cuni.cz

Telephone: +420 224 968 113

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Abstract

The aim of our study was to describe pharmacogenetics of tramadol in paediatric intensive care unit population. The COMFORT-Neo/-B scores were evaluated in neonates and children ≥ three months of age treated with prolonged analgosedation and tramadol dosages and genetic polymorphisms in *CYP2D6, COMT, ABCB1, OPRM1* and *PXR.* Overall, 13 neonates and 14 children were recruited into the study. Higher tramadol doses (mg/kg/h) were found in homozygous vs. heterozygous *ABCB1 rs1045642* subjects [0.26 (0.12 – 0.32 vs. 0.18 (0.13 – 0.3); P = 0.044]. Homozygotes for *ABCB1 rs1045642* were significantly less often undersedated (COMFORT score > 22). No other significant association was found between other single nucleotide polymorphisms and tramadol dosing and COMFORT-Neo/-B score values. *ABCB1 rs1045642* polymorphism potentially contributes to the clinical aspects of tramadol in neonates and children treated in paediatric intensive care unit.

Key words: analgosedation, drug dosing, paediatric intensive care, pharmacogenetics, tramadol

Introduction

Tramadol, antinociceptive drug 6000-times less potent than morphine and 10-times less potent than codeine (1), is considered to be safe and reliable component of analgosedation in neonatal and paediatric intensive care units (2-14). Tramadol is more advantageous than other typical opioid agents for its unique pharmacological profile, since it exhibits a lower incidence of side effects and potential (15, 16). The drug is produced as a racemic mixture with important differences in binding, activity and targets associated with the two enantiomers (17, 18). The (+) enantiomer has higher affinity to opioid receptors and preferentially inhibits serotonin uptake and enhances serotonin release, whereas the (-) enantiomer inhibits norepinephrin reuptake (19).

Tramadol is a substrate of ABCB1 efflux transporter (20) and is extensively metabolized in the liver via cytochrome isoenzymes CYP2D6, and CYP2B6 and CYP3A4. The main metabolites O-demethyltramadol (M1) and N-demethyltramadol (M2) originate via CYP2D6 and CYP3A4 (21-23). The M1 is considered the most pharmacologically active, being up to 6 times more potent than the parent drug in producing analgesia, and 200 times more potent in μ-opioid (OPRM1) binding (24).

Gene polymorphisms in *CYP2D6*, efflux transporter *ABCB1* and target, the μ-receptor *OPRM1*, potentially contribute to variability of tramadol efficacy/safety (25) . It is well recognized that *CYP2D6* gene is highly polymorphic with numerous allelic variants (more than 109 so far), (26) resulting in varying degrees of loss of metabolic activity (extensive, intermediate, and poor metabolizer phenotypes). For *CYP2D6*, this also includes gene duplication as a contributor to an "ultra-rapid" metabolizer (UM) phenotype. In addition, there are clinically significant differences in the frequencies of the variant alleles between ethnic populations (25). The activity of cytochrome P 450 is strongly influenced by transcription factor pregnane X receptor (PXR) and *OPRM 1* polymorphism effect has been described to be associated with polymorphisms in catechol-o-methyl transferase (*COMT*), (27-29).

In a prospective study setting, we aimed to analyze tramadol dosing in context with possible pharmacogenetics covariates in specific conditions of pediatric intensive care unit.

Material and Methods

The study was approved by the Ethics Committee of the General Faculty Hospital (FWA 00003027) and it was conducted in accordance with the Declaration of Helsinki. Artificially ventilated full-term neonates and children treated with tramadol on the pediatric intensive and resuscitation unit in the Clinic of Pediatrics and Adolescent Medicine, General Faculty Hospital were prospectively recruited

into the study from January 2010 until September 2013 after obtaining informed consent from the legal representatives.

Overall 27 Caucasians, neonates ≥ 36 gestational weeks and children ≥ three months of age with length of continuous intravenous infusion of tramadol ≥ 48 hours were included. The principal exclusion criteria included severe hepatic and acute renal failure according to Goldstein and pRIFLE criteria, according to international guidelines (30, 31).

Demographics, clinical data, COMFORT-B, COMFORT-Neo score for analgosedation assessment were recorded (linear weighted kappa median 0.80), (32). Values of COMFORT Neo/-B score > 22 were considered indicative for undersedation and < 11 for oversedation (33).

Samples of peripheral venous blood for DNA isolation were collected in tubes containing EDTA and immediately frozen and stored at - 20 °C until further processing. Genomic DNA was extracted from blood leukocytes using QIAamp DNA Blood Mini Kit (Quiagen Ltd.). Detection of the candidate gene polymorphisms in *COMT (rs4633, rs4680, rs4818) ABCB1 G2677T/A (rs2032582), ABCB1 C3435T (rs1045642), OPRM1A118G (rs1799971)* and *PXR 10799 G/A, PXR -25385 C/T, PXR -24113 C/T, PXR 7635 A/G* (*rs1054191, rs3814055, rs2276706, rs6785049)* was performed as published previously (34-40).

Chi-square test was used for categorical variables. Mann-Whitney test was used for between group median comparison of doses per kg and hour. A *P*-value of less than 0.05 was considered statistically significant.

Results

Baseline parameters of both neonates and children, are summarized in Table I.

Tab. 1: Baseline characteristic parameters of neonates and children.

Parameter	**Neonates (n = 13)**	**Children (n = 14)**
Sex (M/F)	8/5	10/4
PMA median (range)	40.0 (39 - 42)	107 (49 - 730)
Weight (kg), median (range)	3.50 (2.68 – 4.00)	9.73 (3.45 – 50)
Apgar score 1st min, median (range)	4 (0 – 9)	-
Apgar score 5th min, median (range)	7 (0 – 9)	-
Apgar score 10th min, median (range)	7 (0 – 10)	-

F-female, M-male, PMA-postmenstrual age

Study subjects (n = 27 Caucasians, 18 boys and 9 girls) suffered from various causes of perinatal asphyxia RSV pneumonia, aspiration of amniotic fluid, sepsis, meningoencephalitis, status epilepticus, burns, and myocarditis.

Except tramadol, the patients received midazolam (11 neonates, 11 children), sufentanil (nine neonates; 12 children) and phenobarbital (all neonates, 12 children). Some subjects received also clonidine (one neonate and six children), ketamine (four children) and chloralhydrate (four children). No statistically significant difference was found between neonates and children in tramadol dose/kg/h, (0.23 (0.13 – 0.25) vs. 0.27 (0.03 – 0.34) mg/kg/h). COMFORT Neo/-B scores < 11 was registered in more than one third of the observations in neonates, but only rarely in children over three months. On the contrary, only exceptionally score over 22 was registered in neonates (Tab. II).

Tab. 2: COMFORT score values in neonates and children.

Parameter	**Neonates (n = 13)**	**Children (n = 14)**
COMFORT Neo/-B score	11 (2 – 31)	16 (5 – 31)
COMFORT Neo/-B score > 22/n	9/1389	84/1541
COMFORT Neo/-B score < 11/n	446/1389	25/1541

Values in median (range), n-total number of measurements.

The observed allelic frequencies of the studied single nucleotide polymorphisms (SNPs) are shown in Table III.

Tab. 3: Allelic frequencies in the study population.

Gene	**SNP**	**RS number**	**Variant allele frequency**
OPRM1	*A118G*	*rs1799971*	0.11
ABCB1	*C3435T*	*rs1045642*	0.59
ABCB1	*G2677T/A*	*rs2032582*	0.35
PXR	*G10799A*	*rs1054191*	0.15
	C25385T	*rs3814055*	0.39
	C24113T	*rs2276706*	0.39
	A7635G	*rs6785049*	0.61
COMT	*C186T*	*rs4633*	0.54
	C408G	*rs4818*	0.37
	G472A	*rs4680*	0.42
CYP2D6	*2549delA (CYP2D6*3)*	*rs35742686*	Not found
	G1846A, 1707delT	*rs3892097*	Not found
	*(CYP2D6*4, CYP2D6*6)*	*rs5030655*	
	XN		Not found

XN-duplication (amplification) of the gene

Gene polymorphisms *rs1799971* for *OPRM1A118G, rs2032582* for *ABCB1 G2677T/A, rs105419, rs3814055, rs2276706* and *rs6785049* for *PXR* and *rs4633, rs4818* and *rs4680* for *COMT* were not significantly associated with either COMFORT Neo/-B score values or tramadol dosing rate per kg and h. Heterozygotes for *rs1045642* in *ABCB1* received lower tramadol dose than variant homozygotes (0.18 (0.13 – 0.3) vs. 0.26 (0.12 – 0.32) mg/kg/h, P = 0.044), and wild-type homozygotes (0.18 (0.13 – 0.3) vs. 0.29 (0.03 – 0.34) mg/kg/h, P = 0.07).

Wild-type Homozygotes for *rs1045642* in *ABCB1* were significantly more often undersedated. Both wild-type homozygotes for *rs1045642* in *ABCB1* (wild-type; n = 7; COMFORT Neo/-B score > 22/n**;** 28/610) and heterozygotes (n = 8; COMFORT Neo/-B score > 22/n; 36/1114*)* were significantly more often undersedated (COMFORT Neo/-B score > 22) than variant homozygotes (n = 12, COMFORT Neo/-B score > 22/n; 13/1272; P = 0.04; P = 0.02, respectively).

A substantial difference was observed between neonates and children over three months of age in COMFORT Neo/-B score values. While children were mostly within therapeutic range (COMFORT Neo/-B score 11-22), neonates were often oversedated (COMFORT-Neo less than 11), which could have affected our results. The study was conducted briefly after implementation of COMFORT-scores monitoring to daily clinical practice, with appropriate interindividual variability score achieved (Cohen's kappa), without dose adjustment based on observed values, which is intended as a next step of the implementation. Although PICU care providers empirically confirm the experience of oversedation of the patients in their department, we cannot comment this finding with respect to literature data, as there are no data available.

Discussion

Tramadol is a safe analgesic drug and its use in the paediatric setting is well established as reported in the literature, however FDA is so far restrictive regarding patient's safety and more studies on dosage regimen are needed. Nevertheless, its pharmacogenetic background deserves awareness that should be held also to well established molecules, as has been shown with codeine, which is no longer recommended for children under 12 years of age due to uncertainty in CYP2D6 activity. Polymorphisms of *CYP2D6* are the most frequently investigated with respect to tramadol efficacy/safety (41). Limited number of subjects was a greatest limitation of our study. Although we genotyped our patients for *CYP2D6*, the occurrence of polymorphic alleles was negligible. Adult poor metabolizers (PMs) have shown reduced metabolite formation and corresponding minimal pain reduction (25, 42-44). On the other hand, ultrarapid (UMs) and extensive metabolisers (EMs) have higher metabolic clearance of tramadol (up to 14 x in EMs vs. PMs), (19, 45-50) than poor metabolisers (PMs) and patients with moderately rapid metabolism (IMs), (47). Single-dose and short-term studies of tramadol showed a higher peak plasma concentration of the active metabolite and also greater analgesia, greater miosis and a higher incidence of nausea in CYP2D6 UMs as compared with EMs (51, 52). It also seems, that adult CYP2D6 UMs are more likely to experience the adverse effects of tramadol (26). Tramadol increased incidence of nausea in UMs in experimental setting (51), although the results of other studies may be conflicting (50). Nevertheless, several case reports document severe toxicity of tramadol in adult UMs (53-55). In children, Allegaert et al. explains variability of tramadol to *O*-demethyl tramadol (M1) clearance by size, postmenstrual age (52.7% of the variability) and also CYP2D6 polymorphisms (6.4% of the variability). Clearance of M1 seems to be very low in preterm neonates, irrespective of the CYP2D6 polymorphism with subsequent rapid maturation, whereas the studies indicate that the subsequent slope of the increase is dependent on the individual CYP2D6 activity score (56, 57). In the recently

published study of the same authors (n = 295, postmenstrual age 25 weeks to 84.8 years, weight 0.5 - 186 kg), low *CYP2D6* genotype activity was associated with lower M1 formation clearance (25 %) than faster metabolizer genotype, but only 32 % of those with low genotype activity were in the slow metabolizer group. Poor metabolizers comprised 9.8 % of subjects reaching 19.4 % of EM M1 formation clearance (58). Similarly to codein, severe respiratory depression is documented in a child with CYP2D6 ultrarapid metabolism after taking tramadol (1mg/kg) for pain relief related to a day-case tonsillectomy (59).

The role of the SNPs within the efflux pump P-glycoprotein gene *ABCB1* needs to be further thoroughly explored. In our relatively small sample of purely Caucasian population, wild-type homozygotes for *rs1045642* in *ABCB1* were significantly less often undersedated in the overall population. As noted, this result could have been distorted by more frequent oversedation in neonates. Nevertheless, significant difference was observed for tramadol dose/kg/h in carriers of *rs1045642* in *ABCB1* surprisingly (contrary to the theoretical expectations) acquiring lower dose than homozygotes for *rs1045642* in *ABCB1*, whereas the dose was still lower compared to wild-type homozygotes, although statistically non-significant. No difference was apparent for *rs2032582* variant allele. We have previously found that plasma concentration-time profiles of tramadol in adult CYP2D6 poor metabolizers were significantly higher in homozygotes for *rs1045642* in *ABCB1* than in carriers of the wild-type allele, but no difference in VAS score, total tramadol consumption, adverse reactions, need for rescue analgesic medication or verbal description of pain was observed in a clinical study in the overall population of patients after arthroscopy. Importantly, the difference was not investigated specifically in the CYP2D6 PM subpopulation (20). Bastami et al. found some associations between tramadol AUC and *rs1045642, rs2032582* and r*s1128503* for *ABCB1* with the highest values in homozygotes of the variant allele, but only for the 50 mg and not for the 100 mg dose (50). The impact of *ABCB1* SNPs may in theory differ in adults and children, since there are few papers indicative of ABCB1 ontogenetic pattern of expression with increasing with age in

children (60, 61). However, the effect of maturation in context with *ABCB1* polymorphism has not been established so far.

We have found no association between *OPRM1, PXR* and *COMT* polymorphisms and COMFORT Neo/-B score values or tramadol doses in our study. Potential impact of *ABCB1* variant alleles on the drug effects was noted, but its clinical significance remains to be clarified.

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Footnote

The study has been presented at a scientific meeting-The 7th Cogress of Europian Academy of Paediatric Societies (EAPS) in Geneve, 2016 (ESPNIC/EAPS) and is a part of a thesis of the first author.

Conflict of interest

The authors declare no conflict of interest.

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