Physiol. Res. 64 (Suppl. 4): S521-S527, 2015

OPRM1 and *ABCB1* Polymorphisms and Their Effect on Postoperative Pain Relief With Piritramide

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Received September 15, 2015
Accepted October 2, 2015

Summary

Genetic factors may contribute to the differential response to opioids. The aim of this study was to evaluate the association between polymorphisms of μ1-opioid receptor gene *OPRM1* (rs1799971), and P-glycoprotein transporter gene *ABCB1* (rs1045642, rs2032582), and piritramide efficacy under postoperative patient-controlled analgesia (PCA). In 51 patients, *OPRM1* variant was associated with decreased efficacy in early postoperative period evidenced by sum of pain intensity difference in the 0-6 h postoperative period ($SPID_{0-6}$), (F=3.27, p=0.029). Mean (SD) $SPID_{0-6}$ was observed in the *118AA* genotype 22.9 (6.1) mm, which was significantly higher from the *118GG* genotype 10.0 (4.4) mm, p=0.006. The lowest cumulative dose was recorded in *118AA* genotype 19.1 (9.8) mg, which was significantly less than in the *118GG* genotype group 36.6 (6.1) mg, p=0.017. Opioid–induced adverse effects were observed in 11, 30, and 100 % of patients in *118AA*, *118AG*, and *118GG* genotype groups, respectively (p<0.05). Piritramide efficacy and safety was not significantly affected by *ABCB1* (rs1045642, rs2032582) polymorphisms. Variant *OPRM1 118G* allele is associated with decreased acute postoperative pain relief after piritramide. Decreased efficacy leads to higher drug consumption under PCA settings, which however, does not fully compensate insufficient pain relief, but increases incidence of adverse effects.

Key words

OPRM1 • ABCB1 • Piritramide • Single nucleotide polymorphism • Acute pain • Patient-controlled analgesia

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Introduction

Opioids are generally considered as the first line therapy for patients with moderate to severe postoperative pain. The dose of opioid required to achieve sufficient postoperative pain relief is highly variable among patients. The interindividual variation in response to opioids can party be attributed to age, gender, weight (BMI), renal or liver functions. However, as each patient often responds differently to specific opioids, providing adequate analgesia for individual patients without concomitant development of adverse effects is still a major challenge.

Genetic factors may contribute to the variable response to opioids by affecting their pharmacokinetics (drug metabolizing enzymes and transporters) or pharmacodynamics (receptors and signal transduction). Variability of analgesic efficacy of opioids has been previously linked to the *OPRM1 (A118G)* polymorphism in the gene encoding μ1-opioid receptor. Homozygotes for the variant allele *(118GG)* required higher opioid doses to achieve pain relief in some studies (Zhang *et al*. 2005, Chou *et al*. 2006). Although the clinical significance of *ABCB1* polymorphism remains to be established for opioids, P-glycoprotein as the gene product of *ABCB1* is involved in the transmembrane transport of opioids (Dagenais *et al*. 2004). *ABCB1* gene is highly polymorphic in the Czech population (Pechandová *et al*. 2006).

The clinical relevance of potentially important single nucleotide polymorphisms (SNPs) in pharmacokinetics or pharmacodynamics of piritramide has not been studied so far. Piritramide (PIR) has been well established for almost 40 years in postoperative pain management in several European countries (Mueller *et al*. 2006) and is indicated primarily for the treatment of postoperative pain and analgosedation in the ICU setting. It is a 4-amino piperidine derivative (2,2,-diphenyl-4-[1-(4-carbamoyl-4-piperidino)-piperidine]-butyro-nitrile) with an agonistic effect on μ1-opioid receptors and is roughly equipotent to morphine. Metabolic pathways leading to PIR degradation have not been established yet.

In therapeutic doses, hemodynamic and emetic adverse effects are marginal, and compared to morphine, the incidence and extent of side effects such as respiratory suppression and itching are significantly reduced. The aim of this study was to test the association between *OPRM1* **(**rs1799971) and *ABCB1* (rs1045642, rs2032582) polymorphisms on postoperative pain relief with PIR in the acute postoperative period in patients who have undergone elective inguinal hernioplasty.

Materials and Methods

Patients and surgical procedure

This was a prospective study in patients undergoing elective inguinal hernioplasty at the 3rd Department of Surgery, First Faculty of Medicine, Charles University in Prague and University Hospital Motol. The study was approved by the local Ethics Committee and it was conducted in accordance with the Declaration of Helsinki. Main exclusion criteria included allergy to opioids, unwillingness to cooperate in the pain assessment, and administration of non-steroidal anti-inflammatory analgesics and/or opioids one week before the surgery. Standard premedication protocol included diazepam *per os* at the evening before surgery. All patients underwent surgery under standardized general anesthesia, based on the combination of propofol (2 mg/kg) and sufentanil (2 μg/kg). Surgical procedures were conducted by an experienced surgeon, who followed standard inguinal hernioplasty according to Lichtenstein.

Postoperative pain treatment and efficacy analysis

After recovering from the anesthesia, all patients received PIR by intravenous patient-controlled analgesia (PCA). Patients were connected to patient control analgesia pump Infusomat® Space (Braun, Germany) containing 15 mg/50 ml of PIR configured to deliver a bolus dose (0.044 mg/kg) with a 10 min lock-out period. Pain intensity was assessed using visual analogue scale (VAS 0-100 mm) at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 16 h after the surgery. These values were used to calculate the sum of pain intensity differences (SPID). The number of applied PCA bolus doses was assessed at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12 and 16 h after the surgery in patients and the number of boluses was used to calculate the dose of PIR. The pain intensity differences were evaluated during 3 periods 0-6 h, 0-12 h, and 0-16 h and the same intervals are therefore also used to report the cumulative PIR dose and demand. Demand is the dose that represents the number of times the patient pushed the release button of the patient-controlled analgesia. In addition, appearance of opioid-induced adverse effects was recorded. Postoperative nausea and vomiting (PONV) was considered as certain opioid-induced adverse effect, while headache, sedation, pruritus, vertigo, and hallucination represented possible opioid-induced adverse effects. To discriminate from non-specific adverse reactions, only the appearance of either certain opioid-induced adverse effect in individual patient or at least two possible opioid-induced adverse effects resulted in classification as piritramide-induced adverse effect.

Pain intensity, and PIR consumption were compared among the *OPRM1 (A118G)* and *ABCB1 (G2677T, C3435T)* polymorphisms**.**

Genotyping

All samples were coded and all laboratory personal was blinded with respect to any clinical data of the patients. Peripheral venous blood samples were collected in tubes containing K_2EDTA. The samples were immediately frozen and stored at –20 °C until further processing. DNA was subsequently isolated using QIAmp Blood Mini Kit (Qiagen, Germany). Genotyping for *ABCB1 (C3435T, G2677T/A)* polymorphism in MDR1 gene was done as published previously (Pechandová *et al.* 2006). Genotyping of the *OPRM1 (A118G)* polymorphism was done using validated PCR-RFLP method. The primer sequences were 5'-AACACA TACATGACCAGGAAGT and 5'-GGTCAACTTGTCC CACTTAGATC. The amplification of the DNA was done in the termocycler My-Cycler (BioRad, USA). The PCR amplification were conducted in 27 μl reaction volume, containing 60 ng of genomic DNA, 3 μl of

$MgCl_2$ 25 mM (Fermentas, Lithuania), 0.5 µl of 10 mM primer solutions, 2.5 µl of dNTP 2 mM (Fermentas, Lithuania), 2.5 µl of 10 × Biotherm Puffer (Fermentas, Lithuania), 0.15 µl of Tag DNA polymerase (Fermentas, Lithuania) and 15.85 µl of sterile PCR water. The amplification started with an initial denaturation at 94 °C for 2 min followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 1 min and extension at 72 °C for 1 min, followed by 7-min terminal extension at 72 °C. The amplification product was subsequently digested by Bsh1236 I (New England Biolabs, USA) overnight at 37 °C. The fragments were analyzed in a 3.5 % agarose gel dyed by 0.5 µg/ml of ethidium bromide for 5-10 min. The DNA fragments were identified using GelDocum 2000 imaging system (Bio-Rad, USA).

Statistical analysis

Hardy-Weinberg equilibrium was verified for observed genotype frequencies of the *OPRM1 (A118G)* and *ABCB1 (G2677T, C3435T)* polymorphisms and to detect deviation from the expected genotype distribution and to detect genotyping error. Data were expressed as means (standard deviation, SD). Kruskal-Wallis test for two dependent variables was used for statistical evaluation, categorical parameters were evaluated using χ^2 test ($p<0.05$).

Table 1. Demographic and surgical data of patients for *OPRM (A118G)* genotype groups.

Parameter	*118AA* N = 38	*118AG* N = 10	*118GG* N = 3	P value
Age (years)	52.08 (16.26)	57.8 (13.36)	54.0 (18.25)	0.916
Weight (kg)	81.00 (14.78)	77.9 (15.91)	75.66 (8.73)	0.737
Height (cm)	173.94 (9.59)	177.10 (5.76)	174.00 (14.00)	0.667
Gender (male/female)	33/5	7/3	3/0	0.318
Smoking (no/yes)	17/11	6/4	2/1	0.796
Length of operation (min)	56.92 (19.52)	53.4 (21.42)	57.66 (2.51)	0.612
Length of anesthesia (min)	76.65 (19.58)	71.30 (17.29)	77.33 (3.05)	0.515

Data are means (SD).

Table 2. PIR dose, value of SPID and demand in patients with PCA for *OPRM (A118G)* genotype groups.

Parameter	Time interval	*118AA* N = 38	*118AG* N = 10	*118GG* N = 3	P value
Dose (mg)	*0-6 h*	14.49 (6.30)	15.78 (10.99)	19.37 (3.43)	0.255
	0-12 h	18.35 (9.09)	26.27 (18.12)	33.45 (6.26)[a]	0.023
	0-16 h	19.15 (9.81)	31.26 (20.83)[b]	36.56 (6.06)[a]	0.015
SPID (mm)	*0-6 h*	22.86 (6.07)	20.50 (7.39)	10.00 (4.35)[a, c]	0.011
	0-12 h	36.05 (8.83)	37.50 (8.16)	32.33 (3.21)	0.463
	0-16 h	40.02 (9.84)	42.00 (7.98)	38.66 (3.78)	0.502
Demand (mg)	*0-6 h*	15.41 (6.70)	19.73 (13.74)	35.22 (6.25)[a, c]	0.021
	0-12 h	19.03 (7.25)	30.20 (20.82)	44.01 (8.24)[a]	0.012
	0-16 h	19.55 (9.67)	33.61 (22.40)	44.59 (7.39)[a, b]	0.003

Data are means (SD), PIR dose – dose of piritramide, SPID – sum of pain intensity differences, demand is the dose that represents the number of times the patient pushed the release button of the patient-controlled analgesia device. Significant difference between groups ($p<0.05$): [a] *118AA* genotype vs. *118GG* genotype, [b] *118AA* genotype vs. *118AG* genotype, [c] *118AG* genotype vs. *118GG* genotype.

Results

Totally 51 patients completed the study and all have been included into the evaluation. The study population showed considerable interindividual variability with respect to PIR cumulative dose, and SPID. The mean PIR cumulative dose (SD) in the whole study population was 22.55 (13.66) mg (range 3.61-78.14 mg) over the 16 h postoperative period. Mean $SPID_{0-16}$ (SD) was 40.33 (9.19) mm (range 18-60 mm).

Polymorphisms in OPRM1 A118G

The allelic frequency of the variant allele for *OPRM (A118G)* SNP was 15.69 % and the allelic distribution in the study population was in agreement with the Hardy-Weinberg equilibrium. Totally 71.0 % (N=38), 26.5 % (N=10), and 2.5 % (N=3) of the study subjects were classified as wild-type homozygous, heterozygous, and variant homozygous, respectively.

The demographic characteristics were similar across all genotype groups with respect to age, weight, height, sex, smoking history, length of operation and length of anesthesia (Table 1).

Table 2 summarizes analgesic efficacy of PIR in *OPRM (A118G)* genotype groups. The results indicate that patients carrying variant 118G allele (*118GG* genotype and *118AG* genotype) presented significantly higher demand and received significantly higher PIR doses for pain relief compared to the wild-type homozygous patients. The mean cumulative dose during the 0-16 hour's postoperative period was 19.15 mg, 31.26 mg and 36.56 mg among wild-type homozygous, heterozygous, and variant homozygous patient groups, respectively. The differences between the genotype groups with respect to PIR cumulative dose administered during the early 0-6 h after surgery were not statistically significant, although the numerical tendency towards higher dosing in variant allele carriers was similar as in the 6-16 h periods. Lower pain relief was achieved in patients carrying variant allele with the mean (SD) SPID of 10.00 mm (4.35), and 20.50 mm (7.39) in the variant homozygous, and heterozygous subjects, respectively, as compared with wild-type homozygotes 22.86 mm (6.07) in the time interval of 0-6 h.

Table 3. PIR dose, value of SPID and demand in patients with PCA for *ABCB1* (*G2677T, C3435T*) genotype groups.

Parameter	**Time interval**	***2677GG*** **N = 16**	***2677GT*** **N = 26**	***2677TT*** **N = 9**	**P value**
Dose (mg)	*0-6 h*	14.09 (6.56)	15.59 (7.93)	15.09 (7.14)	0.997
	0-12 h	18.40 (11.18)	22.09 (13.49)	21.31 (10.23)	0.746
	0-16 h	19.84 (11.18)	24.11 (15.99)	22.83 (10.46)	0.741
SPID (mm)	*0-6 h*	20.62 (5.79)	22.23 (7.22)	21.77 (5.84)	0.844
	0-12 h	36.18 (9.15)	36.53 (8.86)	36.88 (6.56)	0.968
	0-16 h	40.37 (10.17)	40.11 (9.30)	40.88 (7.91)	0.949
Demand (mg)	*0-6 h*	16.07 (7.95)	18.00 (10.18)	18.16 (11.25)	0.994
	0-12 h	20.26 (11.68)	24.49 (15.65)	23.92 (13.42)	0.724
	0-16 h	20.54 (12.08)	25.10 (17.35)	24.02 (12.91)	0.751
Parameter	**Time interval**	***3435CC*** **N = 10**	***3435CT*** **N = 24**	***3435TT*** **N = 17**	**P value**
Dose (mg)	*0-6 h*	15.53 (6.21)	14.54 (8.30)	15.43 (6.66)	0.647
	0-12 h	20.42 (11.07)	20.23 (14.25)	21.82 (8.98)	0.470
	0-16 h	21.34 (11.77)	22.39 (16.67)	23.48 (10.16)	0.576
SPID (mm)	*0-6 h*	19.80 (5.86)	13.40 (8.51)	21.35 (4.59)	0.603
	0-12 h	35.10 (9.94)	37.95 (8.68)	35.23 (7.29)	0.532
	0-16 h	39.40 (11.26)	41.79 (8.82)	38.82 (8.63)	0.582
Demand (mg)	*0-6 h*	17.77 (7.92)	17.48 (11.75)	17.15 (7.28)	0.688
	0-12 h	22.07 (12.78)	22.76 (17.02)	23.82 (10.17)	0.454
	0-16 h	22.49 (12.78)	23.63 (18.49)	24.09 (10.90)	0.503

Data are means (SD), PIR dose – dose of piritramide, SPID – sum of pain intensity differences, demand is the dose that represents the number of times the patient pushed the release button of the patient-controlled analgesia device.

The ratio of administered/demanded boluses was substantially less (55 %) in variant homozygous patients, as compared with the respective values of 94 %, and 80 % in the wild-type heterozygous patients, and heterozygotes during 0-6 h after surgery.

The rate of opioid-induced adverse effects significantly increased in patient groups carrying variant allele. The incidence of adverse drug reactions was 11 %, 30 % and 100 % in wild-type homozygous, heterozygous, and variant homozygous genotype groups, respectively (p=0.001).

Polymorphisms in ABCB1 G2677T and C3435T

The demographic characteristics were similar across all genotype groups with respect to age, weight, height, sex, smoking history, length of operation and length of anesthesia (data not shown).

The allelic frequency of the variant alleles for *ABCB1 (G2677T/A)* SNP was 43.52 % for allele *T* and 1.96 % for allele *A*. The allelic distribution in the study population was in agreement with the Hardy-Weinberg equilibrium. Approximately 32 % (N=16), 49 % (N=26), and 19 % (N=9) of the study subjects were classified as wild-type homozygous, heterozygous, and variant homozygous, respectively. Heterozygous genotype of *2677GA* was found in two subjects only, while no homozygous carrier of *2677A* allele was detected. Therefore, the impact of *2677A* allele on analgesic consumption, pain intensity as well as PIR medication was not evaluated.

The allelic frequency of the variant allele for *ABCB1 (C3435T)* SNP was 56.86 % and the allelic distribution in the study population was in agreement with the Hardy-Weinberg equilibrium. Approximately 9.5 % (N=10), 25.0 % (N=24), and 16.5 % (N=17) of the study subjects were classified as wild-type homozygous, heterozygous, and variant homozygous, respectively. There were no statistically significant differences in the investigated efficacy parameters among genotype groups of *ABCB1* during 16 h after surgery (Table 3). The incidence of opioid-induced adverse reactions was not associated with the *ABCB1* polymorphisms, although a numerical tendency towards lower incidence among patients carrying variant allele was noted. The incidence was 25 %, 20 % and 12 % in wild-type homozygotes, heterozygotes, and variant homozygotes for *G2677T*, respectively, p=0.061. The respective values for the *C3435T* polymorphism were 30 %, 21 %, and 18 %, p=0.111.

Discussion

In our patient cohort recovering from hernioplasty and receiving PIR *via* PCA, the *OPRM1 (A118G)* genotype-dependent association on PIR efficacy was detected.

Although a number of studies have investigated the association between the *OPRM1 (A118G)* polymorphism and opioid-induced analgesia, a consensus has not yet been reached. It has been suggested that various clinical pain syndromes are not equally affected by a specific pharmacogenetic predisposition. Furthermore, various pain model situations are not equally sensitive to opioid-induced analgesia (Hwang *et al.* 2014). The clinical impact of *OPRM1 (A118G)* variant was recently evaluated in a meta-analysis involving 18 studies and more than 4600 subjects (Hwang *et al.* 2014). Patients, carriers of the variant allele needed higher doses of opioids to achieve sufficient analgesia. These genetic variations were clinically most important in Asian patients, morphine users, and those undergoing visceral organ surgeries. In addition, polymorphisms in other genes coding for drug metabolizing enzymes can influence the response of opioids in a population-specific manner through the changes of blood levels (Ameyaw *et al*. 2001, Saito *et al*. 2006, Bernard *et al.* 2006). Finally, environmental and behavioral effects (e.g. rates of smoking or local dietary habits), may also contribute to variation in efficacy of opioids (Chen *et al*. 2013, Zheng *et al*. 2013).

Individual opioids exhibit different affinities for binding sites, which may determine analgesic capacity. Subgroup analysis by Coulbault *et al.* (2006), Janicki *et al*. (2006) and Zhang *et al*. (2011) suggested that the *OPRM1 (A118G)* polymorphism affected the efficacy of morphine but not fentanyl in acute pain model after colorectal and gynecologic surgery. Our results indicate that the diverse functional selectivity of *OPRM1 (A118G)* polymorphism may also influence the clinical efficacy of piritramide.

There are conflicting results regarding the influence of polymorphism in *ABCB1 (G2677T/A, C3435T)* gene on both effects and side effects of morphine (Fujita *et al.* 2010, Sia *et al*. 2008, Campa *et al.* 2008). It has been suggested that *3435TT* genotype carriers in *ABCB1* responded well to morphine while those with *3435CC* or *3435CT* genotypes responded substantially less. The results from our study did not show any association between genetic polymorphism in

ABCB1 (G2677T, C3435T) gene and analgesic effects of PIR in acute postoperative pain.

There are some limitations of the current study. First, the overall number of patients included in this trial is rather limited. Second, we used cumulative PIR dose administered *via* PCA as the primary outcome and surrogate for pain and analgesic response. However, a fundamental question could be whether one can conclude that an increase in postoperative opioid consumption administered *via* PCA necessarily indicates increased postoperative pain and/or reduced PIR efficacy. This surrogate marker does not take into account other PIR effects, such as euphoria. In theory, subjects might use more opioids because they feel euphoric regardless of pain levels. This may be reflected in the observed increase in PIR use in one group compared with the other, rather than an increased requirement for analgesia. However, we consider this situation unlikely in our study, since the PIR cumulative dose and SPID as a direct efficacy parameter were inversely related among the genotype groups. Thus, the patient group that reported the less pain relief also demanded and consumed the highest cumulative PIR doses, which does not indicate that PIR-induced euphoria was the principal component of motivation for the patients to demand the drug. Third, we did not monitor PIR plasma concentrations in order to determine the drug pharmacokinetics and possible pharmacokinetic factors that could influence its efficacy. The metabolism and elimination of the PIR is not well explored, but the drug elimination is considered to depend almost exclusively on hepatic metabolism, while renal elimination is negligible (Bouillon *et al*. 2004). Since the drug metabolism pathways are unknown, possible involvement of polymorphic drug metabolizing enzymes cannot be excluded and these unknown factors could confound the study results.

Nevertheless, the current study used a model of the acute pain originating from a standardized surgical procedure under the condition of PCA. Patients with the *OPRM1* variant homozygous genotype (*118GG* genotype) required almost twice as much of PIR to achieve pain control at 16 h after surgery than those with the wild-type homozygous (*118AA* genotype). *OPRM1 (A118G)* variants were also associated with the lower values of the percentage ratio of the administered and demanded boluses compared to the *118AA* genotype wild type homozygous group in the 0-6 hour's time interval. This ratio can give us an idea about the lower efficacy of the therapy.

Therefore, we conclude that the identification of *OPRM1 (A118G)* polymorphism may provide valuable information on the individual analgesic doses of PIR required or help to decide on the need for combination analgesic treatment combining PIR with non-opioid medication to achieve satisfactory pain control.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

This study has been supported by Charles University project PRVOUK P25/LF1/2 and UNCE 204022.

Abbreviations

DNA, deoxyribonucleic acid; ICU, intensive care unit; MDR1, multidrug resistence 1; OPRM1, opioid receptor μ1; PCA, patient-controlled analgesia; PCR-RFLP, polymerase chain reaction – restriction fragment length polymorphism; PIR, piritramide; PONV, postoperative nausea and vomiting; SNP, single-nucleotide polymorphism; SPID, sum of pain intensity differences; VAS, visual analogue scale.

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