The association of monoamine oxidase B functional polymorphism with postoperative pain intensity

Omar Šerý [1], Olga Hrazdilová [2], William Didden [3], Věra Klenerová [4], Radim Štaif [1], Vladimír Znojil [5] & Pavel Ševčík [2]

[1] Laboratory of neurobiology and molecular psychiatry, Institute of Biochemistry, Masaryk University, Faculty of Science, Kotlářská 2, 611 37 Brno, Czech Republic;
[2] Department of Anaesthesiology, Masaryk University, Faculty of Medicine, Brno, Czech Republic;
[3] GHC Research Institute, Houston, TX, USA.;
[4] Institute of Medical Biochemistry, 1st Medical faculty, Charles University, Praha;
[5] Department of Pathological Physiology, Masaryk University, Faculty of Medicine, Brno, Czech Republic

Correspondence to: O. Šerý, Ph.D.,
Laboratory of neurobiology and molecular psychiatry,
Institute of Biochemistry, Faculty of Science, Masaryk University,
Kotlářská 2, 611 37 Brno, CZECH REPUBLIC
TEL. + 420 549 497 312; FAX. + 420 542 213 827
EMAIL: omarsery@sci.muni.cz

Submitted: April 25, 2006 *Accepted: May 10, 2006*

Key words: **association; MAOB; pain; polymorphism; gene**

Neuroendocrinol Lett 2006; **27**(3):333–337 **PMID:** 16807522 NEL270306A03 © Neuroendocrinology Letters **www.nel.edu**

Abstract

OBJECTIVES: The monoamine oxidase B (MAO-B) is an enzyme involved in metabolism of dopamine, benzylamine, phenylethylamine, tyramine and tryptamine. The A/G polymorphism in intron 13 of the MAO-B gene has been previously found to be associated with variability of the MAO-B enzyme activity. The aim of the present association study has been to examine the relationship between the A/G polymorphism in intron 13 and postoperative pain intensity.

METHODS: 284 subjects (105 males and 179 females) undergoing planned tonsillectomy were examined. The intensity of pain was evaluated using 100-mm visual analogue scale (VAS). A PCR method with allele specific primers for detection of A/G polymorphism was used.

RESULTS: We found a relationship between the A/G polymorphism in intron 13 of the MAO-B gene and average intensity of postoperative pain in male subjects. Higher average intensity of postoperative pain was detected in males with the G allele (3.96) in comparison with males with the A allele (3.45) and the difference was statistically significant (p<0,03).

CONCLUSIONS: Results of this study indicate the relationship between the MAO-B polymorphism and postoperative pain intensity in the Czech male population. A potential role of MAO-B in the perception of pain intensity is discussed.

ORIGINAL ARTICLE

Abbreviations & Units

AIPP	– average intensity of postoperative pain
COMT	– catechol-o-methyltransferase
CSS	– computer statistic software
DNA	– deoxyribonucleic acid
dNTP	– deoxyribonucleotide triphosphate
IMAO-A	– inhibitor of monoamine oxidase A
IMAO-B	– inhibitor of monoamine oxidase B
L-DOPA	– 2-amino-3-(3,4-dihydroxyphenyl)-propanoic acid
MAO	– monoamine oxidase
OPRM1	– mu-opioid receptor
PCR	– polymerase chain reaction
PEA	– phenylethylamine
TRPV1	– vanniloid receptor subtype 1
UV	– ultraviolent
VAS	– visual analogue scale
h	– hour
bp	– base pair
kg	– kilograms
µg	– micrograms
µl	– microliters
µM	– micromols
M	– mols
min	– minute
mM	– milimols
mm	– milimeters
ng	– nanograms
s	– second

Introduction

Recent progress in molecular biology has enabled gene expression modulations in animal models using techniques of "knock-out", "oligo-antisense" and viral vectors. First association studies using PCR method investigate the impact of so-called candidate genes on pain perception emerged in the last two years. The methods mentioned above thus enable investigations, at a molecular level, of which out of the approximately 30 thousand genes of the human genome may be involved in pain mediation, which of these are polymorphic and which polymorphisms are responsible for interindividual differences in pain perception.

Except for congenital pain insensitivity and familiar hemiplegic migraine, no mutations which would themselves (monogenically) cause pain perception modulation have been found. Variation in the pain sensitivity is not caused by one gene. It is evident that pain sensitivity represents an integrated feature of a polygenic character and is caused by interaction of several genes. Association studies can be used to study such polygenic characters [1].

Some association studies of pain perception have already been published. Pharmacogenetic studies represent a paper published by Klepstad et al. [10]. This study found a statistically significant association between A118G polymorphism of the opioid receptor µ gene (OPRM1) and efficiency of morphine in pain treatment of cancer patients. Patients with the GG genotype needed more morphine to cope with pain than heterozygotes or the AA homozygotes. Fillingim et al. [3] reported an association of A118G single nucleotide polymorphism of the mu-opioid receptor gene (OPRM1) with pressure pain sensitivity. Kim et al. [8] investigated interactions among variability in pain sensitivity, sex, ethnicity, temperament and gene polymorphisms in vanniloid receptor subtype 1 (TRPV1), δ opioid receptor subtype 1 and catechol-o-methyltransferase (COMT) genes. Experimentally evoked pain was measured using a visual analogue scale (VAS). TRPV1 and δ opioid receptor subtype 1 polymorphisms were statistically significantly associated with pain sensitivity. Association studies focused on pain research have recently been reviewed [17].

Monoamine oxidase (MAO, EC1.4.3.4) catalyzes oxidative deamination of many neurotransmitters and dietary amines. It is a membrane-bound mitochondrial enzyme that exists in two forms – MAO-A and MAO-B. The MAO-A preferentially catalyzes the oxidative deamination of serotonin, norepinephrine and epinephrine; the MAO-B preferentially catalyzes the oxidative deamination of benzylamine and phenylethylamine. Dopamine, tyramine and tryptamine are common substrates for both forms of the enzyme. Genes for both forms of the enzyme are located on the X chromosome. Garpenstrand et al. [4] studied the influence of the A/G polymorphism in intron 13 of the MAO-B gene on MAO-B activity and they found that individuals with the "A-allele" displayed significantly lower enzyme activity than individuals with the "G-allele" ($p=0.019$).

Altered levels of MAO-B have previously been associated with a number of psychiatric and neurological conditions, e.g. schizophrenia, depression, alcoholism and neurodegeneration. Plasma MAO-B levels were found to be increased in depressed patients [9]. Platelet monoamine oxidase activity was shown to correlate inversely with human personality traits such as sensation seeking and impulsiveness [12].

Our study was focused on an investigation of the association between the A/G polymorphism in intron 13 of MAO-B gene and postoperative pain perception. Patients undergoing planned tonsillectomy were used as a model of pain.

Materials & Methods

Subjects

284 patients (178 women and 106 men) of Caucasian population undergoing planned tonsillectomy in the years 2002–2004 were included in the association study. This study was approved by the Ethical Committee of the Faculty Hospital of Saint Anna in Brno. All patients signed an Informed Consent.

Patient experience with pain was evaluated prior to operation. Patients that underwent surgery up to two times but without polytrauma were included in the study. Individuals with diagnosed diabetes mellitus type I and II, hypertensive patients and patients with tumours were excluded from the study. Patients who experienced polytrauma, psychiatric patients with depressive and anxiety disorders and persons treated with psychoactive drugs during the last ten years were neither included in the study. Patients treated with non steroid anti-inflam-

matory drugs and with corticoids were also excluded due to possible biased postoperative pain perception.

The surgery was carried out under total anaesthesia. Sufentanil was dosed 25 μg for patients with weight up to 60 kg and 35 μg for patients with weight over 60 kg, being applied at least 30 minutes before the end of surgery. The intensity of pain was evaluated using 100-mm visual analogue scale (VAS). Postoperative pain was treated with metamizol in the individual dose of 1g applied by slow infusion. The first analgesic dose was applied as soon as the pain intensity reached the value 30 of the VAS. The pain intensity was monitored with the VAS for the first time right after patient's extubation and then every 60 minutes in the course of 5 hours' postoperative period. Venous blood samples of 2 ml were collected from all patients preoperatively.

Methods

Individual genomic DNA samples were extracted from blood using the chemagic Blood100 Kit (Chemagen, Germany). MAO-B genotypes were determined by the allele specific polymerase chain reaction (PCR). The PCR was performed using primers described by Garpenstrand et al. [4]. The used primer sequences were A-specific 5´- CAC TGG CAA ATA GCA AAA GT -3´, G- specific 5´- CAC TGG CAA ATA GCA AAA GC -3´ and forward primer 5´- GGA TTT ACT TTG CAG GCA CC -3´. Amplification reactions were carried out in a volume of 50 μl, containing 100 ng of genomic DNA, 0.5 M of each dNTP, 5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.4), 50 mM KCl, 1 μM of each primer and 1 unit of Taq-Purple DNA polymerase (Top-Bio, Czech Republic). After an initial denaturation at 94°C for 2.5 min, DNA was amplified in three-step cycles as follows: denaturation at 94°C for 30 s, annealing at 59°C for 30 s and extension at 72°C for 30 s, using the Techne Gradient thermal cycler (Techne, England). After 32 cycles, a final extension time of 7 min was applied at 72°C. The length of the amplified fragment was 663 bp. The PCR products were analyzed with agarose gel electrophoresis on a 2% agarose gel EliPhore (ELISABETH PHARMACON, Czech Republic) containing ethidium bromide and were then visualized under UV light.

Results

All 284 subjects were genotyped for the A/G polymorphism in intron 13 of the MAO-B gene. Table 1 presents the genotyping data. After division of all subjects into subgroups by sex, a statistically significant difference in average intensity of postoperative pain between genotypes A and G in male subjects was observed (p=0.021). Male subjects with G genotype referred higher average intensity of postoperative pain (VAS score = 3.96) than male subjects with A genotype (VAS score = 3.45). No statistically significant difference in the average intensity of postoperative pain between the two genotypes of MAO-B gene was observed in female subjects nor in all subjects without sex differentiation.

Discussion

Our observation suggests that the average intensity of postoperative pain could be influenced by functional polymorphism of the MAO-B gene in male subjects only. Males with the G genotype reported higher average intensity of postoperative pain (VAS score = 3.96) than males with the A genotype (VAS score = 3.45). The allele G with higher activity was associated with higher average intensity of postoperative pain.

Kurth et al. [11] detected A/G polymorphism in intron 13 of the MAO-B gene by a single-strand conformational polymorphism analysis. Garpenstrand et al. [4] demonstrated the relationship between platelet MAO-B activity and the A/G polymorphism in intron 13 of the MAO-B gene. Individuals with the "A-allele" displayed a significantly lower enzyme activity than individuals with the "G-allele" [4].

The lack of effect of the A/G polymorphism in intron 13 of MAO-B gene on intensity of postoperative pain in females might be caused by a higher level of estrogens and by inhibition effect of estrogens on MAO-B activity in female subjects. High levels of estrogen have been related to increased feelings of well-being and low levels of depression. Some evidence suggests that these beneficial effects of estrogen may involve its influence on MAO. Antidepressant activity of estrogens may be performed via inhibition of the MAO pathway [2]. Estrogen therapy reduces plasma MAO activity in postmenopausal woman

Table 1. Average intensity of postoperative pain (AIPP) in A/G polymorphism in intron 13 of MAOB gene

Subjects	**Totally**	**Genotype**		
		AA (A)	AG	GG (G)
Males	105	53	–	52
AIPP	3,71 (±1,31)	3,45 (±1,28)*	–	3,96 (±1,31)*
Females	179	63	56	60
AIPP	3,56 (±1,52)	3,60 (±1,55)	3,39 (±1,44)	3,68 (±1,57)

*Statistically significant difference (p<0,03)

with depressed mood [6]. Holschneider et al. [7] suggest on the basis of experiments on rats that estrogen exerts a tissue-specific, differential regulation of MAO-A and MAO-B activity.

The relationship between the MAO-B gene and intensity of postoperative pain could have multiple reasons. MAO-B activity influences levels of substrates like dopamine, benzylamine, phenylethylamine, tyramine and tryptamine. These substrate levels can consequently influence modulation of some neurotransmissions.

Firstly, dopamine could be involved in the effect of the MAO-B gene on pain feeling. The striatum and striatal dopamine D2 receptors are involved in the regulation of behavior [16], as well as in the regulation of pain in humans [5]. Painful stimulation produces an increase of the regional cerebral blood flow in the human striatum. Positron emission tomography findings show that low dopamine D2 receptor availability in the striatum of healthy subjects (indicating either a low density of dopamine D2 receptors or a high synaptic concentration of dopamine) is associated with a high cold pain threshold and a low capacity to recruit central pain inhibition by conditioning stimulation [5]. L-DOPA relieves pain after conversion to dopamine, with the dopamine sedating pain transmission in the way of the dopamine D2 receptor [18]. In our study, pain related to higher activity allele G of MAO-B was found. This could show lower dopamine extracellular level. We excluded the possibility of explaining our results by MAO-B influence on pain intensity by means of the dopamine level.

The potential role of phenylethylamine in mood state, which could influence pain feeling, is even more interesting. Phenylethylamine (PEA), another substrate of MAO-B, is an endogenous neuroamine which increases attention and activity in animals. PEA has been shown to relieve depression in 60% of depressed patients. It has been proposed that PEA deficit may be the cause of common form of depressive illness. PEA improves mood as rapidly as amphetamine but does not produce tolerance [14]. The current knowledge indicates that brain phenylethylamine may be a neuromodulator of aminergic synapses and that it promotes energy, elevates mood, and favors aggression. Phenylacetic acid, the main metabolite of PEA, is decreased in the biological fluids of depressed subjects and schizophrenic subjects and is increased in schizoaffective subjects. The administration of PEA or its precursor L-phenylalanine improves mood in depressed patients treated with a selective monoamine oxidase B inhibitor [13]. Szabo et al. [19] studied whether aerobic exercise affects phenylacetic acid concentration in the urine and they found that 24 hour mean urinary concentration of phenylacetic acid was increased by 77% after exercise. As phenylacetic acid concentration in urine reflects phenylethylamine level, which is known to have antidepressant effects, phenylethylamine may be linked to the therapeutic effects of physical exercise on depression. In our study we detected higher activity allele G of MAO-B gene in subjects that felt higher postoperative pain and this could be influenced by a lower PEA level.

Sanchez-Blazquez et al. [15] studied imidazoline compounds in the modulation of morphine analgesia. The effects of highly selective imidazoline ligands on the supraspinal antinociception induced by morphine in mice were determined. Pre-treatment (30 min) with deprenyl, an irreversible inhibitor of monoamine oxidase B (IMAO-B), produced an increase in morphine antinociception. Clorgyline, an irreversible IMAO-A, applied 30 min prior to morphine did not alter the effect of the opioid. During longer intervals (24 h), a single dose of either clorgyline or deprenyl reduced the density of I(2)-imidazoline receptors and prevented the I(2)-mediated potentiation of morphine analgesia. In our study, a lower activity allele A of MAO-B in subjects with lower pain intensity could also influence the sufentanil (opiate) analgesia.

Subjective interpretation of pain intensity using VAS may cause potential imprecision in our data. We are aware that this can be a limiting factor in the interpretation of the results and a larger number of patients as well as other models of pain must be used to verify our results.

Statistics

The relationship between A/G polymorphism in intron 13 of the MAO-B gene and postoperative pain intensity was assessed in a case-control study. The statistical software CSS Statistica (Statsoft, Tulsa, USA) was used. The average intensity of postoperative pain was tested using the Mann-Whitney U Test.

Acknowledgments

This study was supported by the grant no. NM/7146 – 3/2002 of the Internal Grant Agency of the Ministry of Health of the Czech Republic (IGA MZ CR) and by the project No. MSM0021620806.

REFERENCES

1 Belfer I, Wu T, Kingman A, Krishnaraju RK, Goldman D, Max MB. Candidate gene studies of human pain mechanisms: methods for optimizing choice of polymorphisms and sample size. Anesthesiology 2004; **100**:1562–1572.
2 Chakravorty SG, Halbreich U. The influence of estrogen on monoamine oxidase activity. Psychopharmacol Bull 1997; **33**:229–233.
3 Fillingim RB, Kaplan L, Staud R, Ness TJ, Glover TL, Campbell CM, Mogil JS, Wallace MR. The A118G single nucleotide polymorphism of the mu-opioid receptor gene (OPRM1) is associated with pressure pain sensitivity in humans. J Pain 2005; **6**:159–167.
4 Garpenstrand H, Ekblom J, Forslund K, Rylander G, Oreland L. Platelet monoamine oxidase activity is related to MAOB intron 13 genotype. J Neural Transm 2000; **107**:523–530.
5 Hagelberg N, Jaaskelainen SK, Martikainen IK, Mansikka H, Forssell H, Scheinin H, Hietala J, Pertovaara A. Striatal dopamine D2 receptors in modulation of pain in humans: a review. Eur J Pharmacol 2004; **500**:187–192.
6 Holsboer F, Benkert O, Demisch L. Changes in MAO activity during estrogen treatment of females with endogenous depression. Mod Probl Pharmacopsychiatry 1983; **19**:321–326.
7 Holschneider DP, Kumazawa T, Chen K, Shih JC. Tissue-specific effects of estrogen on monoamine oxidase A and B in the rat. Life Sci 1998; **63**:155–160.
8 Kim H, Neubert JK, San Miguel A, Xu K, Krishnaraju RK, Iadarola MJ, Goldman D, Dionne RA. Genetic influence on variabil-

ity in human acute experimental pain sensitivity associated with gender, ethnicity and psychological temperament. Pain 2004; **109**:488–496.
9 Klaiber EL, Broverman DM, Vogel W, Kobayashi Y. Estrogen therapy for severe persistent depressions in women. Arch Gen Psychiatry 1979; **36**:550–554.
10 Klepstad P, Rakvag TT, Kaasa S, Holthe M, Dale O, Borchgrevink PC, Baar C, Vikan T, Krokan HE, Skorpen F. The 118 A > G polymorphism in the human micro-opioid receptor gene may increase morphine requirements in patients with pain caused by malignant disease. Acta Anaesthesiol Scand 2004; **8**:1232–1239.
11 Kurth JH, Kurth MC, Poduslo SE, Schwankhaus JD. Association of a monoamine oxidase B allele with Parkinson's disease. Ann Neurol 1993; **33**:368–372.
12 Oreland L, Hallman J. The correlation between platelet MAO activity and personality: short review of findings and a discussion on possible mechanisms. Prog Brain Res 1995; **6**:77–84.
13 Sabelli HC, Javaid JI. Phenylethylamine modulation of affect: therapeutic and diagnostic implications. J Neuropsychiatry Clin Neurosci 1995; **7**:6–14.
14 Sabelli H, Fink P, Fawcett J, Tom C. Sustained antidepressant effect of PEA replacement. J Neuropsychiatry Clin Neurosci 1996; **8**:168–171.
15 Sanchez-Blazquez P, Boronat MA, Olmos G, Garcia-Sevilla JA, Garzon J. Activation of I(2)-imidazoline receptors enhances supraspinal morphine analgesia in mice: a model to detect agonist and antagonist activities at these receptors. Br J Pharmacol 2000; **130**:146–152.
16 Sery O, Drtilkova I, Theiner P, Pitelova R, Staif R, Znojil V, Lochman J, Didden W. Polymorphism of DRD2 gene and ADHD. Neuroendocrinol Lett 2006; **27**: 236–240.
17 Sery O, Hrazdilova O, Matalova E, Sevcik P. Pain research update from genetic point of view. Pain Practice 2005; **5**:341–348.
18 Shimizu T, Iwata S, Morioka H, Masuyama T, Fukuda T, Nomoto M. Antinociceptive mechanism of L-DOPA. Pain 2004; **110**:246–249.
19 Szabo A, Billett E, Turner J. Phenylethylamine, a possible link to the antidepressant effects of exercise? Br J Sports Med 2001; **35**:342–343.