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

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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.
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. Pharmagenetic 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 vanillloid 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 benzyllamine 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-inflamm-
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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 TGGCAA ATA GGA AAA GT -3´, G- specific 5’- CAC TGGCAA ATA GGA 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 MgCl₂, 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.


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.
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, benzyamine, 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 and tryptamine. These substrate levels can consequently influence modulation of some neurotransmissions.

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 (MAO-B), produced an increase in morphine antinociception. Clorgyline, an irreversible MAO-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.

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