The lack of association between monoamine oxidase (MAO) intron 13 polymorphism and platelet MAO-B activity among men

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Abstract: Monoamine oxidase (MAO), a mitochondrial flavine containing enzyme, exists in two isoenzymes, MAO-A and MAO-B. Platelets contain MAO-B subtype, proposed to be a biomarker for different personality characteristics and vulnerability for substance abuse. The most common polymorphism of MAO-B gene, a single base change (A or G) occurs in intron 13. It has been proposed to be a functional polymorphism, controlling the activity of MAO-B in platelets. The aim of the study was to determine the association between platelet MAO-B activity and MAO-B intron 13 polymorphism in 225 racially and ethnically uniform healthy Caucasian men of the Croatian origin. Our results showed that platelet MAO-B activity did not differ between subjects subdivided into those with «A allele» or «G allele». This polymorphism of the MAO-B gene did not control the activity of the MAO-B in platelets. Platelet MAO-B activity was associated only with the smoking status, and it was significantly decreased in smokers when compared to nonsmokers. No significant association was found between MAO-B polymorphism and smoking status. In healthy individuals of the Croatian origin, the studied MAO-B polymorphism showed a lack of functional importance in regulating MAO-B activity in platelets. Since different populations may vary in the association between functional polymorphism and the MAO-B activity, and the genotype of transcription factor AP-2β was reported to be associated with altered platelet MAO-B activity, and with specific personality traits, further studies on different populations should be conducted to elucidate the molecular mechanism/s regulating platelet MAO-B activity.

Keywords: Monoamine oxidase (MAO), platelets, MAO-B activity, MAO-B intron 13 polymorphism, Control male subjects

Introduction

Monoamine oxidase (MAO) is a mitochondrial flavine containing enzyme. There are two types of MAO: MAO-A and MAO-B. Both catalyze the deamination of different neurotransmitter and xenobiotic amines in the central nervous system and in the periphery. Platelets contain only MAO-B, which shares the identical amino acid sequence with MAO-B in central nervous system (Billett, 2004; Oreland, 2004). The main substrates for MAO-B are exogenous monoamines such as phenyletylamine and benzylamine, as well as dopamine and tyramine. Two forms of MAO isoezymes differ in their substrate and inhibitor specificity and are encoded by the separate genes. Both genes are located side-by-side on the chromosome X (Xp11.23-11.4), have 15 exons and have identical exon-intron organization. MAO-B gene is 60 Kb long. The corresponding protein has 520 amino acids. Both MAO isoforms share a 70% amino acid sequence homology.

Platelet MAO or MAO-B has been proposed to be a biomarker for different personality disorders, including aggressive behavior, sensation seeking behavior, monotony avoidance, and vulnerability to psychiatric disorders and substance abuse (Schalling et al., 1987; Garpenstrand et al., 2000; Oreland, 2004). MAO-B activity in platelets has been reported to be affected by various factors, such as sex, age, ethnicity, and smoking (Garpenstrand et al., 2000; Oreland et al., 2002; Fowler et al., 2003; Oreland, 2004; Costa-Mallen et al., 2005a).

The most common polymorphism of MAO-B gene occurs in polymorphic repeat region of the intron 13, and includes a single base change (A or G), (Girmen et al., 1992). The association between MAO-B genotype and MAO-B activity in platelets has been proposed, linking lower enzyme activity with «A-allele» in blood (Garpenstrand et al., 2000). However, the opposite results have been reported, showing no association between MAO-B genotype and MAO-B activity in platelets (Girman et al., 1992), or lower enzyme activity in subjects with «G-allele» in postmortem human brains (Balciuniene et al., 2002). Since smoking decreases platelet MAO-B activity (Fowler et al., 2003), we divided our subjects according to the smoking status. To elucidate the association between platelet MAO-B activity and MAO-B genotype (i.e. G/A substitution on intron 13), and to avoid the influence of sex and ethnicity on platelet MAO activity and on the frequency of MAO-B alleles (Costa-Mallen et al., 2005a), we determined simultaneously platelet MAO-B activity and MAO-B genotype in a large group of racially and ethnically uniform healthy Caucasian men from Croatia.

Materials and methods

Subjects

The study included randomly selected 225 male Caucasian healthy subjects of the Croatian origin, 41.97 ± 12.36 years old (range 20-65 years). Participants were healthy blood donors (Transfusion Clinic, Zagreb, Croatia), with no personal or family history of psychopathology, free of neurodegenerative or psychiatric disorders, and no medical treatment. All participants fulfilled the questionnaire answering the questions about their medical history, drinking and smoking habits.

All participants agreed to give a blood sample, to participate in the study, and gave their written informed consent. The study was approved by the Ethic committee.

Biochemical analysis

Blood (4 ml) was drawn from cubical vein at 8.00 a.m. in a plastic syringe with 1 ml of acid citrate dextrose (ACD) anticoagulant. Platelet-rich-plasma (PRP) was obtained by centrifugation (935 x g) for 70 s at room temperature. Platelets were sedimented by further centrifugation of PRP at 10,000 x g for 5 min. The platelet pellet was washed with saline and centrifuged again. Platelets were destroyed by sonication (20 kHz, amplitude 8 x 10^{-3} mm for 30 sec). Platelet MAO-B activity was determined by the spectrofluorimetric method, using kynuramine as a substrate, by a slight modification of the method of Krajl (1965), as previously described (Pivac et al., 2002). Briefly, specimens of standard, blank (water) and platelet sonicates (100 µl) were analyzed in duplicates in phosphate buffer (0.5 M, pH 7.4), incubated 60 min on 37° C with kynuramine. The reaction was stopped by adding ice-cold 1N NaOH. The measurement of 4-hydroxyquinoline (4-HOQ) fluorescence, a product of kynuramine, was performed on a Varian Cary Eclipse spectrofluorimeter, with an exciting wavelength of 310 nm and emitted wavelength of 380 nm. Platelet protein was determined by the method of Lowry et al. (1951).

The DNA analysis

Genotyping methods were performed in our laboratories in Division of Molecular Medicine at the Rudjer Boskovic Institute. DNA was isolated from blood by a standard salting out method. DNA samples were genotyped for the MAO-B intron 13 G/A polymorphism in ABI Prism 7000 Sequencing Detection System apparatus (ABI, Foster City, USA) using Taqman-based allele-specific polymerase chain reaction assay, according to the procedure described by the Applied Biosystems. The primers and probes were purchased from Applied Biosystems (ABI, Foster City, USA).

Statistical methods

All data (expressed as mean ± standard deviations) were evaluated by one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Two-way ANOVA was used to test the significance of the several variables: smoking, genotype, and age, on platelet MAO activity, and to test the interactions between them. The correlation between parameters was determined by Pearson's correlation coefficient. The frequency of smokers and nonsmokers in subjects with particular genotypes (A or G) was determined by a χ^2 test. Hardy Weinberg equilibrium was tested with a χ^2 test. The significance was accepted when $P<0.05$.

Results

The subjects were divided according to the smoking status into 161 nonsmokers and 64 smokers. Platelet MAO-B activity (nmol 4OHQ/mg protein/h) differed significantly [$F(1,223)= 12.462$, $P<0.001$, one way ANOVA] between smokers

(12.54 ± 6.52) and nonsmokers (15.53 ± 5.40), with significantly ($P<0.05$, Tukey's test) lower platelet MAO-B activity in smokers than in nonsmokers. Platelet MAO-B activity was reduced by 19.3% in smokers when compared to nonsmokers.

To evaluate the influence of the MAO-B intron 13 polymorphism on platelet MAO-B activity, we divided subjects according to those with "A-allele" or "G-allele". One-way ANOVA showed no significant [$F(1,223)=0.421$, $P=0.517$, one-way ANOVA] difference in platelet MAO-B activity (nmol 4OHQ/mg protein/h) between 124 subjects with "A-allele" (14.91 ± 6.09) and 101 subjects with "G-allele" (14.40 ± 5.65). The MAO-B polymorphism was in the Hardy Weinberg equilibrium ($\chi^2 = 0.985$, df = 1; $P=0.321$).

Fig. 1 shows that smoking status significantly [$F(3,223)= 4.409$, $P=0.005$, one-way ANOVA] affected platelet MAO-B activity in smokers and nonsmokers subdivided according to the "A-allele" or "G-allele" (Fig. 1). Smokers with "A-allele" or "G-allele" had significantly ($P<0.05$, Tukey's test) lower platelet MAO-B activity than nonsmokers with "A-allele" or "G-allele". Platelet MAO-B activity did not differ significantly ($P>0.05$, Tukey's test) within smokers with "A-allele" or "G-allele", or within nonsmokers with "A-allele" or "G allele" (Fig. 1). The frequency of the occurrence of smokers and nonsmokers was not significantly different ($\chi^2 = 0.0058$, df = 1; $P=0.939$, Odds Ratio= 1.166, 95% CI = 0.06493- 2.092) in subjects with "A-allele" or "G-allele".

To further evaluate the effect of MAO-B polymorphism ("A-allele" or "G-allele") and smoking status (smoking or nonsmoking) on platelet MAO-B activity, and to test the interaction between these variables, two-way ANOVA was used. It revealed a significant main effect [$F(2,221)=3.168$, $P=0.044$], significant effect of smoking [$F(1,221)=5.894$, $P=0.016$], but no significant effect of MAO-B polymorphism [$F(1,221)=0.338$, $P=0.567$], and no significant interaction between smoking and MAO-B polymorphism [$F(1,221)=0.830$, $P=0.373$] on platelet MAO-B activity. When age was tested as a covariate, two way ANOVA showed that age of the subjects did not affect significantly [$F(1,220)=1.751$, $P=0.187$] platelet MAO-B activity.

To determine whether platelet MAO-B activity correlates with age of the subjects, or with the number of cigarettes smoked per day, Pearson's correlation coefficient was used. There was no significant correlation between platelet MAO-B activity and age of the subjects in 64 smokers ($r=0.058$; $P=0.649$) or 161 nonsmokers ($r=-0.05$; $P=0.529$). No significant correlation was found when smokers were subdivided into 37 subjects with "A-allele" ($r=-0.022$; $P=0.987$) or 27 subjects with "G-allele" ($r=0.168$; $P=0.402$), or when nonsmokers were subdivided into 87 subjects with "A-allele" ($r=0.001$; $P=0.990$) or 74 subjects with "G-allele" ($r=-0.118$; $P=0.318$), respectively.

Number of smoked cigarettes per day was not significantly correlated with platelet MAO-B activity in 37 smokers with "A-allele" ($r=-0.224$; $P=0.145$) or 27 subjects with "G-allele" ($r=-0.013$; $P=0.951$).

Discussion

In a well characterized, large group of ethnically and racially uniform, Caucasian healthy male subjects of the Croatian origin, free of neurodegenerative or psychiatric disorders, and without medical treatment, platelet MAO-B activity did not differ between subjects subdivided according to the polymorphism in intron 13 of the MAO-B gene, into subjects with «A allele» or «G allele». Although the association between MAO-B genotype and MAO-B activity in platelets has been proposed (Garpenstrand et al., 2000; Balciuniene et al., 2002), but also disputed (Girman et al., 1992), our results showed comparable values of platelet MAO-B activity in subjects carrying «A allele» or «G allele». These data indicate that this polymorphism of MAO-B gene does not control the activity of the MAO-B in platelets. In contrast to our data, it has been reported that subjects carrying «A allele» have lower platelet MAO-B activity (Garpenstrand et al., 2000), and the authors proposed that MAO-B polymorphism represents a functional polymorphism associated with altered platelet MAO-B activity. The discrepancies between studies might be explained with the slightly different method used to identify the polymorphism in intron 13 (A/G) of the human gene encoding MAO-B, by the different populations used (Balciuniene et al., 2002), and the fact that our study, as opposed to study of Garpenstrand et al. (2000) with 55 male nonsmokers, included much larger groups (i.e. 161 nonsmokers and 64 smokers). Our results are in line with the lack of any correlation between MAO-B polymorphism and platelet MAO-B activity in 70 healthy male nonsmokers (Filic et al., 2005), obtained on the same population, although this group used slightly

different methods for the determination of platelet MAO-B activity and MAO-B polymorphism.

As expected, platelet MAO-B activity was considerably associated with the smoking status, and it was significantly increased in male nonsmokers when compared to male smokers. Smoking is a factor contributing to a lower MAO-B activity (Fowler et al., 2003; Pivac et al., 2005), and decreased binding of 3H-lazabemide to MAO-B in the right amygdaloidal complex has been found in postmortem brains of the smoking subjects (Karolewitz et al., 2005). It is assumed that factors in the cigarette smoke might be associated with development of addiction (Fowler et al., 2003). Smoking is one of the most important problems in public health, a major risk for mental health, and patients with different psychiatric disorders are often heavy smokers. The possible neurobiological basis of smoking, i.e. the effect of nicotine from the tobacco smoke, involves the release of the brain dopamine, noradrenalin or serotonin, and the inhibition of the activity of MAO-A and MAO-B (Fowler et al., 2003), linking smoking with other forms of addictions, and/or psychiatric disorders (Berlin et al., 1995). In line with a proposed inhibitory effect of smoking on platelet MAO-B activity (Berlin et al., 1995; Oreland et al., 2002; Oreland, 2004; Pivac et al., 2005), we have found a significant decreasing effect of tobacco smoke on platelet MAO-B activity in healthy male subjects.

Although the dopaminergic pathways play a significant role in addiction (such as tobacco consumption) and award, and therefore it might be expected that MAO-B polymorphism is important for the control of smoking behavior, we found no

significant differences in platelet MAO-B activity between smokers with "A-allele" or "G-allele", or between nonsmokers with "A-allele" or "G-allele", and no significant interaction between smoking and MAO-B polymorphism. In addition, the frequency of the occurrence of tobacco consumption did not differ in subjects with "A-allele" or "G-allele". No significant association was found between MAO-B intron 13 polymorphism and smoking status in control men or women (Costa-Mallen et al., 2005a). Our data are in line with the lack of significant effect of the genetic variants in dopamine metabolic enzymes (MAO-A and MAO-B and dopamine-β-hydroxylase) on the tobacco consumption in the large sample (Johnstone et al., 2002). Since the genetic predisposition to smoking is determined by multiple genes, recently the particular combination of the genotypes for MAO-B and dopamine D2 receptor genes was found to be associated with a higher risk for smoking behavior in men (Costa-Mallen et al., 2005a).

In our study platelet MAO-B activity was not correlated with the number of cigarettes smoked per day, a finding in line with no relationship between the metabolite of nicotine in plasma, cotinine concentration, and platelet MAO-B activity (Fowler et al., 2003). Other factors from the cigarette smoke, norharman and harman, two substances belonging to the β-carboline alkaloid family, have been shown to reversibly inhibit human MAO-A and MAO-B activities (Herraiz and Chaparro, 2005). Since β-carboline alkaloids are pharmacologically active compounds affecting serotonergic, benzodiazepine, opioid and imidazoline receptors, and might exert antidepressant-like effects, this might explain some "neuroprotective" effects of smoking, e.g. lower incidence of Parkinson's disease

in smokers who have decreased platelet MAO-B activity (Herraiz and Chaparro, 2005).

Platelet MAO-B activity is under influence of sex, and sex differences are found in platelet MAO-B activity, with higher values of platelet MAO-B in female than in male subjects (Muck-Seler et al., 1991; Verkes et al., 1998; Snell et al., 2002; Oreland, 2004; Coccini et al., 2005; Pivac et al., 2005). To avoid the influence of sex and ethnicity on platelet MAO-B activity and on the frequency of MAO-B alleles (Costa-Mallen et al., 2005a), present study included only male control subjects. The inclusion criteria of only healthy men, who fulfilled the questionnaires about their medical status, excluded the possible influence of the different disorders such as pernicious anemia, Alzheimer's disease, Parkinson's disease and Huntington's chorea (Oreland, 2004), that have been shown to increase platelet MAO-B activity. Although older age has been associated with increased values of MAO-B in platelets (Nicotra et al., 2004), and in brain (Fowler et al., 2003; Karolewitz et al., 2005), but also the lack of significant effect of age on platelet MAO-B activity has been observed (Coccini et al., 2005), we did not find any significant correlation between platelet MAO-B activity and age of the subjects either in smokers or in nonsmokers. In addition, age as a covariate did not affect significantly platelet MAO-B activity in the large groups of control male subjects in our study, presumably because our 225 healthy male blood donors were 42 years old (the dispersion of the age data was less than 2%), and slight nonsignificant age-related increase of platelet MAO-B activity was detected only in the older male subjects than the group used in our study (Coccini et al., 2005).

Platelet MAO-B activity is reported to be associated with personality traits such as impulsiveness, extroversion, sensation seeking, neuroticism, or novelty seeking (Kozaric-Kovacic et al., 2000; Oreland et al., 2002; Oreland, 2004), impulse and affect dysregulation (Verkes et al., 1998), and might represent a biological marker for the personality traits that increase the vulnerability for drug-abuse, social mal-adaptation or dysinhibitory psychopathology (Oreland et al., 2002; Oreland, 2004; Longato-Stadler et al., 2002). The mechanism controlling the enzyme activity is still not clear, and our (present study) and other data (Johnstone et al., 2002) do not support the hypothesis that MAO-B polymorphism with a single base change (A or G) on the intron 13, is a functional one, i.e. that this single polymorphism regulates MAO-B activity. Our data are not in contradiction with the results obtained on the postmortem brain tissue, where "A-allele" was significantly associated with elevated MAO-B activity in the brain, when compared to individuals that have "G-allele" (Balciuniene et al., 2002). Namely, although MAO-B in blood and brain shares the identical amino acid sequence (Coccini et al., 2005), MAO-B is expressed in a cell- and tissue-specific manner (Billett, 2004 Costa-Mallen et al., 2005b), no association was found in the activity of MAO-B in platelets and the brain (Winblad et al., 1979), and therefore, MAO-B might be regulated differently in the blood and brain (Ekblom et al., 1998). In addition, different populations may vary in the association between functional polymorphism and the enzyme activity (Balciuniene et al., 2002).

In conclusion, our results have shown that MAO-B polymorphism (G/A substitution in intron 13) showed a lack of functional importance in regulating

MAO-B activity in platelets in a large group of ethnically and racially uniform, healthy Causation male subjects of the Croatian origin. Since the genotype of transcription factor AP-2β was reported to be associated with altered platelet MAO-B activity (Damberg et al., 2000a), and with specific personality traits (Damberg et al., 2000b), further studies on different populations (Balciuniene et al., 2002), with large groups of subjects, should be conducted to elucidate the molecular mechanism/s regulating platelet MAO-B activity.

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Fig 1. Platelet MAO-B activity (nmol 4OHQ/mg protein/h) in healthy male subjects of the Croatian origin, subdivided according to the smoking status (smokers and nonsmokers) and MAO-B polymorphism (subjects with "A-allele" and subjects with "G-allele"). Numbers in the parenthesis are the number of subjects. Results are means ± standard deviations. $^{*}P<0.05$ vs. the values in the corresponding nonsmokers (Tukey's test).