Platelet Monoamine Oxidase B Activity in "de novo" and L-Dopa Treated Parkinsonian Patients and Controls

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ABSTRACT: ***Objective:*** Previous studies demonstrated controversial results regarding monoamine oxidase B (MAO-B) activity in platelets in the periphery in parkinsonian patients (PD). ***Subjects and Methods:*** Therefore we determined platelet MAO-B activity in three age- and sex-matched groups of 17 untreated, so called "de novo" patients with Parkinson's disease (PD), 17 parkinsonian patients, receiving levodopa, and 17 controls by a radio enzymatic assay. ***Results:*** No significant differences of MAO-B activity appeared. ***Conclusion:*** This result suggests that phenotypic determination of MAO-B activity in platelets may not be used as peripheral marker in PD and that levodopa treatment does not alter MAO-B activity in the periphery.

RÉSUMÉ: Activité de la monoamine oxydase B plaquettaire chez les patients naïfs et traités par la L-dopa et chez les contrôles. ***Objectif:*** Les résultats des études antérieures concernant l'activité de la monoamine oxydase B (MAO-B) plaquettaire périphérique chez des parkinsoniens (MP) sont controversés. ***Sujets et méthodes:*** Nous avons donc mesuré l'activité de la MAO-B plaquettaire par dosage radio-enzymatique chez 3 groupes de 17 patients appariés quant à l'âge et au sexe, soit 17 parkinsoniens jamais traités par la L-dopa, 17 parkinsoniens recevant de la L-dopa et 17 contrôles. ***Résultat:*** Il n'y avait pas de différences significatives dans l'activité de la MAO-B. ***Conclusion:*** Ce résultat suggère que la détermination de l'activité de la MAO-B plaquettaire ne peut être utilisée comme marqueur du patient dans la MP et que le traitement par la L-dopa ne modifie pas l'activité périphérique de la MAO-B.

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Various enzymes, such as catechol-O-methyl transferase or monoamine oxidase (MAO) metabolise biogenic amines, e.g., dopamine, in the brain. MAO can be differentiated biochemically and pharmacologically into two forms (types A and B) with different substrate specificities and inhibitor sensitivities. Animal studies have indicated that MAO-A is mainly, but not exclusively, located in brain neurons, such as neurons of the locus coerulus, while MAO-B is preferentially found in serotonergic neurons, glia and astrocytes.[1,2] Elevated levels of MAO-B were found in the substantia nigra of parkinsonian patients. Estimation of peripheral blood cell activities of monoamine oxidase B may reflect central enzyme activity. Therefore platelets were often used as source for MAO-B estimation in the periphery in *in vivo* studies.[3,4] MAO-B is potentially relevant to an oxidative stress model of Parkinson's disease (PD) causation, because catabolism of catecholamines via monoamine oxidase B (MAO-B) may generate hydrogen peroxide.[5] Hydrogen peroxide is converted to highly reactive hydroxyl radicals after reaction with transition metals.[6] Free radical formation and disturbed detoxification processes may be essential contributors to the pathogenesis of Parkinson's Disease (PD).

The aim of this study was to investigate, whether chronic levodopa application may induce changes of MAO-B activity, because enhanced dopamine metabolism might contribute to or even trigger oxidative stress.[7] We determined platelet MAO-B activity in three age- and sex-matched groups of untreated, so-called "de novo" patients with PD, parkinsonian patients receiving levodopa without further antiparkinsonian medication, and controls.

Material and Methods

Subjects

Seventeen untreated, so called "de novo" idiopathic caucasian parkinsonian patients (mean age: 63.9, SD:10.5, range: 51-83 years, 9 females and 8 males, HYS:1.88 SD: 0.60, range 1-3), 17 patients with idiopathic PD, only treated with l-dopa/benserazide or carbidopa for at least four weeks without any further antiparkinsonian medication (mean age: 67.4, SD: 8.0; range: 50-77 years; 8 females and 9 males, HYS: 2.76, SD:

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1.25, range 1-5, daily levodopa dosage: 338.2, SD: 164.2; range: 75-750 mg) and 17 controls (mean age: 63.9, SD:10.5; range: 50-83 years, 9 females, 8 males) were enrolled into the study. Selegiline was withdrawn for at least four weeks in subjects treated with selegiline. Subjects taking drugs with a known influence on mitochondrial function or MAO-B activity in platelets, or smokers were excluded.

125 mg l-dopa/benserazide or carbidopa was applied at 8 a.m. in treated parkinsonian patients. Blood of all subjects was drawn at 8.30 a.m.

Separation of Blood Platelets

Preparation of blood platelets was performed according to Koide et al.[3] 20 ml of venous blood were collected into 5% potassium EDTA containing tubes. Platelet-rich plasma (PRP) was prepared by centrifugation at 4°C at 170 g for 20 min and separated from other blood cells. Platelets were counted in a separated aliquot of the PRP. The remaining PRP was centrifuged at 2700 g for 15 min, the resulting pellet was resuspended in 500 µl NH_4Cl (0.87%). After subsequent centrifugation at 1500 g for 10 min the pellet was washed with saline (0.9%), resuspended in 500 µl 0.25 M sucrose and frozen at -70°C. Prior to performing the radio enzymatic assay the thawed pellet was disrupted by sonication (Bransson Sonifier, 20 kHz) with a microprobe with five pulses at 5 W, 4°C. The amount of protein was assayed with bovine serum albumin as standard (Bio-Rad, Munich, Germany).[8]

Measurement of Platelet MAO-B Activity

MAO-B activity in platelets was assayed according to a modified method of Wurtman and Axelrod[9] and evaluated according to Tipton and Youdim.[10] Platelets (500 µg protein) were preincubated in 0.1 M phosphate buffer, pH 7.4 (total volume 225 µl) for 7 min at 37°C. The reaction was started by addition of 25 µl 0.1 M phosphate buffer pH 7.4, containing (1-14 C-ethyl)-phenylethylamine (PEA, New England Nuclear, Boston, USA; 100 µM; specific activity 2.2 GBq/mmol). After 7 minutes of incubation 250 µl of 1 N HCl were added. The acidified solution was extracted with 2 ml of ethyl acetate by shaking for 10 min. The two phases were separated by centrifugation at 1000 g for 5 min and 1 ml of the organic phase containing the desaminated metabolites was combined with 4 ml biosolve cocktail (Roth, Karlsruhe, Germany) and counted in a liquid scintillation counter (Beckmann Instruments, Munich, Germany). Recovery of labeled products amounted to 98% of total radioactivity used. All assays were performed in triplicate. Values were corrected for blank activity. MAO-B activity was measured as pmol of products formed/mg protein/min. PEA was used in a final concentration of 10 µM with an activity of 5.2 kBq/assay. Choosing these conditions MAO-B activity was linear with respect to time and protein content.

Statistics

One-way analysis of variance (ANOVA) was used for comparison of MAO-B activity in all three groups, linear regression (r^2) and Spearman rank analysis (r) for correlations, using the two-tailed approach.

Results

No significant differences (ANOVA $F_{(dF\ 2,\ dF\ 48)} = 0.33$, $p = 0.72$) between MAO-B activity of untreated "de novo" parkinsonian patients (mean: 362.4 pmol/mg/min, SD: 76.5, range: 269.0-495.2), treated parkinsonian patients (mean: 360.8 pmol/mg/min, SD: 94.3, range: 150.4-516.0) and controls (mean: 384.7 pmol/mg/min, SD: 111.0, range: 236.1- 612.0) were found (Figure). A significant influence of age in controls ($r^2 = 0.267$, $p = 0.033$), "de novo" patients ($r^2 = 0.296$, $p = 0.023$) in contrast to treated parkinsonian patients ($r^2 = 0.103$, $p = 0.208$) appeared. No association between HYS score and MAO-B activity appeared in treated ($r = 0.412$, $p = 0.11$) and "de novo" ($r = 0.38$, $p = 0.123$) parkinsonian subjects. No influence of sex was found.

Discussion

Previous studies on MAO-B activity in platelets in parkinsonian patients and controls showed controversial results. Mann et al.[11] found no differences. Zeller et al.[12] indicated lower (not significant) MAO-B activity in PD, using tyramine as substrate. Significantly increased activity of MAO-B in untreated[13-15] and treated (mainly levodopa/benserazide and amantadine sulfate) parkinsonian patients[16] were found, measuring with phenylethylamine (PEA). With dopamine and kynuramine significant reductions of MAO-B activity in untreated parkinsonian patients were demonstrated.[17-20] Therefore one has speculated, that the number of hydroxyl groups of the used substrate might influence the estimation of peripheral MAO-B activity. Nevertheless the controversial results of peripheral enzyme MAO-B activity in PD were also referred to the different genotypes of MAO-B, because they may cause altered affinities for the used substrates of the assay and/or they may appear in different frequencies in various populations.[12-14,17-20] Studies on the frequency of MAO-B genotypes also showed controversial results. A polymorphism in intron 13 of the MAO-B gene was identified and it was speculated that one allele occurs more frequent in PD compared to controls.[21] Significantly different frequencies of allelic polymorphisms of the MAO-B appeared in PD.[22] However a more recent study did not confirm this result.[23]

In our study untreated, "de novo" and only levodopa treated caucasian[11-23] parkinsonian patients were included in order to

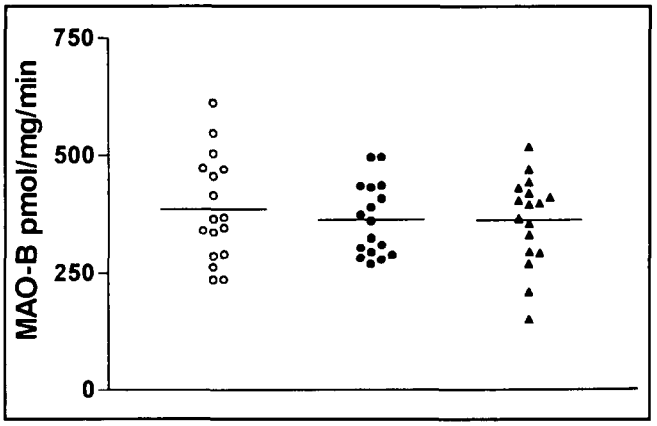

Figure: Legend MAO-B enzyme activity values of (○ controls, ● "de novo" and ▲ levodopa-treated parkinsonian patients, - mean value.

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demonstrate possible effects of levodopa therapy on peripheral MAO-B activity. No significant differences of MAO-B activity appeared in all three investigated groups in this study. This implies as well, that various genotypes of MAO-B have no essential effect on MAO-B enzyme activity.

According to our results, chronic levodopa application does not essentially affect peripheral MAO-B activity. But due to the positive correlation with age in controls and "de novo" parkinsonian patients in contrast to the lack of association with age in the case of levodopa treated parkinsonian patients, one may speculate that a slight effect of levodopa on MAO-B activity may be responsible for this lack of association.

In conclusion we postulate, that platelet MAO-B enzyme activity is not a marker for PD, and is not affected by levodopa therapy. The discussed overrepresentation of one genotype of the MAO-B enzyme in PD[21-23] might not influence peripheral phenotypic enzyme activity of MAO-B.

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