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Abstract Patients with Parkinson's disease receive selective irreversible monoamine oxidase (MAO)-B inhibitors, but their effects on MAO-A activity are not known during long-term application. We determined MAO-A inhibition in plasma samples from patients with MAO-B inhibitor intake or without MAO-B inhibitor treatment and from healthy controls. We detected a 70 % reduction of MAO-A activity in patients with MAO-B inhibitor therapy in comparison to the other groups. Our results suggest that treatment with MAO-B inhibitor may also influence MAO-A activity in vivo, when administered daily.

Keywords Parkinson's disease · MAO-A · MAO-B inhibitors · Selegiline · Rasagiline

Introduction

Treatment with selective monoamine oxidase (MAO)-B inhibitors (MAO-B-Is) such as selegiline and rasagiline is common in patients with Parkinson's disease (PD) (Deftereos et al. 2012). Two isoforms of the enzyme, MAO-A and MAO-B, are known according to MAO (EC 1.4.3.4) substrate and inhibitor specificities (Johnston 1968). MAO-A is primarily located in the periphery with approximately 80 % of total MAO activity in the gastrointestinal tract, lung and placenta. MAO-B is the major isoform in the brain, blood platelets and abundant in the basal ganglia (Collins and Youdim 1970). Serotonin (5-HT), noradrenaline, adrenaline, dopamine as well as β-phenylethylamine are major MAO substrates. Loss of dopamine, noradrenaline and 5-HT is one essential biochemical indicator of the neurodegenerative process in PD (Bernheimer et al. 1973). In order to prolong the duration of action of L-Dopa, inhibitors of L-Dopa decarboxylation (Birkmayer and Mentasti 1967) as well as inhibitors of MAO-B (Birkmayer et al. 1975) and COMT (Napolitano et al. 1995) have been implemented in the treatment of PD. To date, the effect of chronic MAO-B-I application on MAO-A activity in humans is not well known. Results of a PET study showed that daily oral 10 mg selegiline administration inhibited MAO-A after 28 days in the striatum of healthy individuals (Fahn et al. 2011). In this

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study, we investigated the impact of repeated MAO-B-Is application on MAO-A enzyme activity in plasma of PD patients for the first time.

Materials and methods

Collection of plasma samples

Plasma samples from PD patients treated with MAO-B-Is, from PD patients without MAO-B-Is and from healthy controls (clinical characteristics see Table 1) were isolated from 10 ml EDTA blood. Blood samples were taken at 11 am, 4 h after the oral intake of rasagiline or selegiline. The blood was stored on ice and after centrifugation the plasma fraction was aspirated and stored at −80 °C.

Determination of MAO-A enzyme activity

MAO-A enzyme activity was measured using MAO-Glo™ Assay (#V1401, Promega, Mannheim, Germany), which provides a homogeneous luminescent method for MAO-A. 12.5 µl of plasma was diluted in specific MAO-A reaction buffer and added to each well containing 120 µU of MAO-A enzyme (#V1452, Promega, Mannheim, Germany). No additional selegiline (Sigma Aldrich, Schnelldorf, Germany) was added to the wells with plasma samples collected from patients. The control experiment was performed with standardized plasma (Sigma Aldrich, Schnelldorf, Germany) with different doses of selegiline (10 nM–10 mM) to determine IC_{50}. Positive controls contained no plasma and no selegiline, only the MAO-A enzyme. The protocol was done according to the manual (Promega, Germany), just the incubation time was adapted to 50 min at 37 °C. The end measurement was carried out in a fluorescence multi plates meter at an excitation wavelength of 542 nm and an emission of 590 nm.

Table 1 Demographic data of Parkinson's disease (PD) patients and controls

	MAO-B-Is-treated PD patients	No MAO-B-Is-treated PD patients	Healthy controls
Age (years; MW ± STD)	66 (±7)	73 (±6)	66 (±14)
Gender (f/m)	12/12	6/6	3/6
Treatment (rasagiline/selegiline)	16/8	–	–
Treatment duration (days; MW ± STD)	167.5 (±271)	–	–
Doses Rasagiline/selegiline	1 mg/5 mg	–	–
Total number	24	11	9

MAO-B-Is monoamine oxidase-B inhibitors, *MW* mean, *STD* standard error of mean, *mg* milligram, *m* male, *f* female

Statistical analysis

Groups were compared by one-way analysis of variance (ANOVA) in combination with the post hoc Scheffé Test and $p < 0.05$ was considered as significant. We normalized the results by subtracting the plasma quench effect from raw data and converted the data into percentages. The MAO-A enzyme activity without plasma and selegiline has been set at 100 % and the healthy control group (no PD) was set at 100 % (Fig. 1). The presented results were calculated against MAO-A activity or healthy controls, and represent at least three repeated experiments with four internal repeats.

Legal issues and ethical conduct

All subjects gave written informed consent. An independent local institutional review board approved this study. The open observational study was advertised according to § 4 Abs. 23 Satz 3 AMG at the medical association.

Results

Plasma samples taken from PD patients treated with MAO-B-Is showed a reduction of more than 70 % of the MAO-A enzyme activity compared to patient samples without MAO-B-Is therapy and healthy controls (Fig. 1a). Owing to the fluorometric-based assay and the high concentration of plasma proteins, which may divert the emitted light, the plasma samples quenched the MAO-A activity in general (−64 %) (Fig. 1b). We took this effect into account, but could still demonstrate a distinct decline of MAO-A activity in the MAO-B-Is-treated patients (Fig. 1a). There were no differences between rasagiline- and selegiline-treated PD patients (Fig. 1a). Gender and age had no influence on MAO-A activity (data not shown). In addition, the direct effects of selegiline on MAO-A activity were tested in vitro (Fig. 1b). After incubating the human control standardized plasma samples (obtained from Sigma Aldrich) with 10 µM selegiline or higher doses, the MAO-A enzyme activity decreased (12–30 %) compared to a negative control (H_2O). In this experiment, the IC_{50} of selegiline was determined at 44 µM (data not shown) for MAO-A. So far the IC_{50} of selegiline was determined just for MAO-B at 8 nM (Saura et al. 1992).

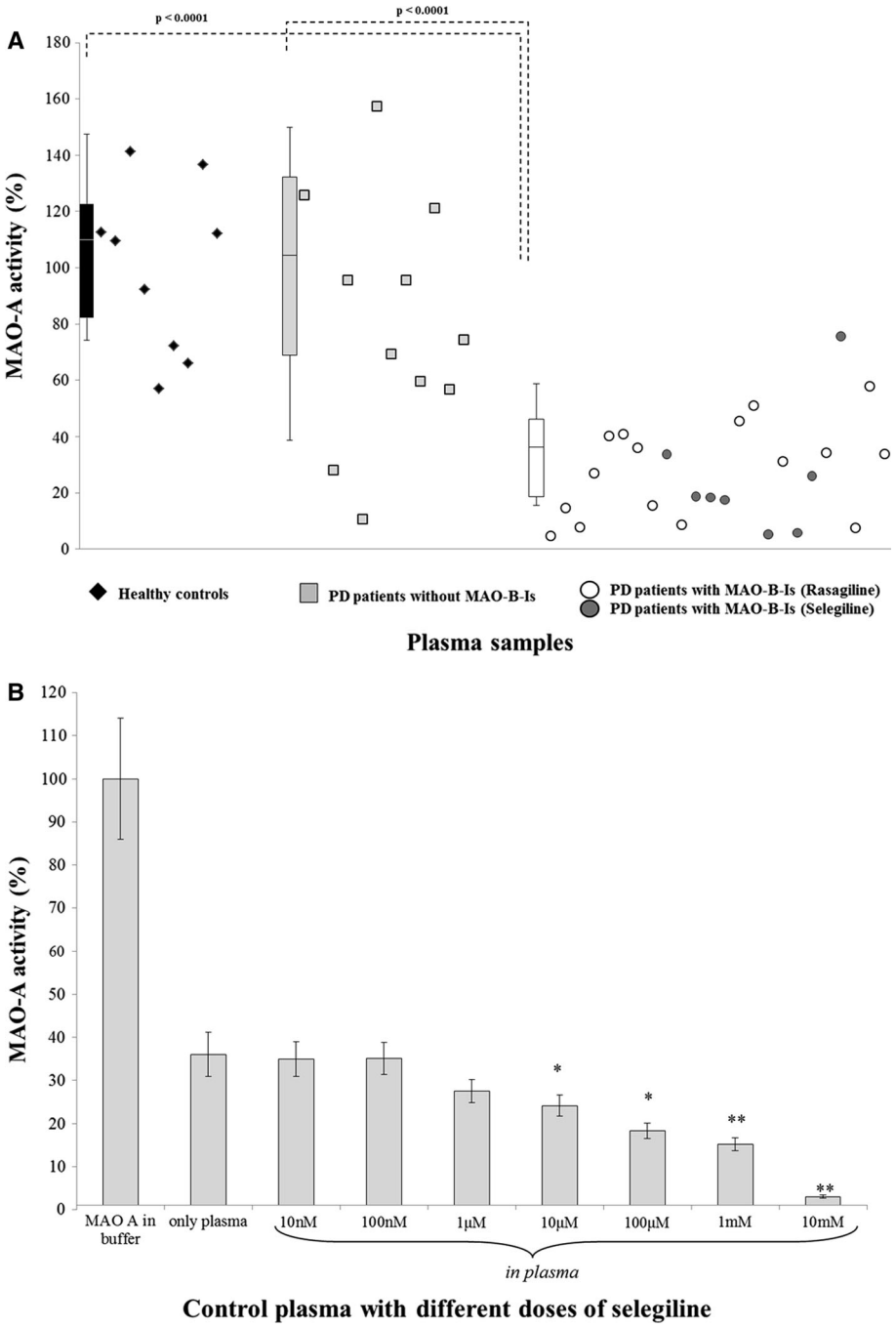

Fig. 1 **a** Monoamine oxidase-A (MAO-A) activity in plasma samples. Plasma samples of healthy controls and Parkinson's disease (PD) patients with and without MAO-B inhibitors (MAO-B-Is) treatment were tested. The MAO-A enzyme activity was measured via MAO-Glo™ Assay. Procedure and measurement was done according to the manual. The *box plots* show the sample minimum, lower quartile, median, upper quartile and samples maximum of each investigated group. Statistical analysis was done using one-way ANOVA with the Scheffé Test for post hoc analysis and the measurement was repeated four times with three internal repeats. The healthy controls (no PD) were set as 100 % [absolute mean value = 1.631.030 ± 101.812 relative luminescent units (RLU)] and compared to PD patients without MAO-B-Is (absolute mean value = 1.393.674 ± 178.485 RLU) and to PD patients with MAO-B-Is (absolute mean value = 428.500 ± 37.200 RLU). *Dashed lines* indicate $p < 0.001$. Healthy controls, $n = 9$; PD patients without MAO-B-Is treatment, $n = 11$; PD patients with MAO-B-Is treatment, $n = 24$. **b** Plasma quenches assay signal of MAO-A activity. Control plasma samples were incubated with different doses of selegiline and afterwards measured via MAO-Glo™ Assay. Procedure and measurement was done according to the manual. Statistical analysis was done using one-way ANOVA with post hoc Scheffé Test and the experiment was repeated three times with three internal repeats. The statistical analysis was done as a comparison of standard plasma without any treatment to different selegiline doses. $*p < 0.05$; $**p < 0.01$; $n = 3$

Discussion

For the first time, we could demonstrate a 70 % reduction in MAO-A enzyme activity ex vivo in plasma from PD patients treated with selective irreversible MAO-B-Is. Pharmacokinetic considerations may partially explain these results. The mean elimination half-life time ($t_{1/2}$) of selegiline is 1.5 h; the $t_{1/2}$ of rasagiline is 1.0 h in controls and 1.3 h in patients (Chen et al. 2007; Mahmood et al. 1995). A fourfold increase of $t_{1/2}$ to 6.0 h was demonstrated after chronic selegiline administration in a daily dose of 10 mg (Mahmood 1997). Since we drew plasma samples 4 h after MAO-B-Is intake, we hypothesize that active drug in plasma was responsible for the described MAO-A inhibition. Our findings are supported by experimental studies in rats treated with 5 mg/kg selegiline orally for 5 days. Animals were decapitated 2 h after the last application. Bowel MAO-A and MAO-B inhibition was 84 and 85 %, respectively. Liver MAO-B inhibition was 92 %, while MAO-A was blocked by 32 %. In the brain tissue, MAO-B inhibition was 92 % and MAO-A was inhibited by 18 % (Magyar 2011). However, subcutaneously and thus first-pass effect circumventing application of 5 mg/kg selegiline for 5 days inhibited bowel MAO-B at 80 % and MAO-A at 62 %, liver MAO-B at 91 % and MAO-A at 34 %, while brain MAO-B was blocked by 99 % and MAO-A by 85 % (Magyar 2011). This study clearly shows differences of MAO inhibition in the peripheral and central nervous systems following MAO-B-Is treatment, but is in line with the suggestion that "selective" MAO-B-Is inhibit MAO-A activity according to dosage. This finding is supported by a PET study using ^{11}C-clorgyline to assess MAO-A activity in individuals with either high-dose orally integrated tablet selegiline (ODT-S; Zelapar®) compared to a transdermal formulation of selegiline (EMSAM) (Fahn et al. 2011). The authors demonstrated that ODT-S (10 mg/day) inhibits brain MAO-A to a similar degree than EMSAM (6 mg/day) (Fahn et al. 2011). These findings and those of Magyar (2011) demonstrate that the selegiline concentration after high oral doses or when administered subcutaneously or via transdermal application is high enough to inhibit MAO-A activity. Our study is in line with these findings and demonstrates that the concentration of both, selegiline (5 mg/daily) and rasagiline (1 mg/daily), in plasma 4 h after the last application is high enough to inhibit MAO-A activity. According to Magyar (2011), this effect is already seen after short-term (5 days) treatment of rats. Therefore, accumulation of selegiline or rasagiline or their metabolites with putative MAO-A/B inhibiting properties may not explain this effect. Since long-term treatment with selegiline increases the $t_{1/2}$ of the drug (Mahmood 1997; Chen et al. 2007), this may keep the concentration of the inhibitor high enough to block MAO-A in addition to MAO-B. Indeed, 70 % inhibition of MAO might be sufficient to increase MAO-A substrate concentration (Green et al. 1977). But this potential loss of MAO-B-Is specificity does not have to be a clinical disadvantage. An inhibition of both isoenzymes suggests reducing neurotransmitter amine degradation, biosynthesis of ROS and other toxic compounds (Riederer et al. 2004, 2007).

In conclusion, we showed that chronic MAO-B-I application also inhibits MAO-A activity ex vivo and may therefore affect both peripheral and brain MAO. However, a better understanding of the regulatory mechanisms of MAO-B-Is and further research on the role of MAO-A/-B interactions in the pathology of neurodegenerative disorders such as PD as well as in the clinical concepts of "disease modification" and neuroprotective capacity of MAO-B-Is is warranted (Mousseau and Baker 2012; Jenner and Langston 2011; Naoi et al. 2012).

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Conflict of interest The authors declare neither competing financial interests regarding this research project nor conflicts of interest with respect to the content of the article.

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