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DOI: <https://doi.org/10.1097/WNF.0000000000000233>

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ZORA URL: <https://doi.org/10.5167/uzh-138330>

Journal Article

Published Version

Originally published at:

Müller, Thomas; Riederer, Peter; Grünblatt, Edna (2017). Determination of Monoamine Oxidase A and B Activity in Long-Term Treated Patients With Parkinson Disease. *Clinical Neuropharmacology*, 40(5):208-211.

DOI: <https://doi.org/10.1097/WNF.0000000000000233>

Determination of Monoamine Oxidase A and B Activity in Long-Term Treated Patients With Parkinson Disease

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Background: Biogenic amines and monoamine oxidase inhibitors influence peripheral monoamine oxidase enzyme activity in chronic levodopa/dopa decarboxylase inhibitor-treated patients with Parkinson disease. Rasagiline is an irreversible inhibitor of monoamine oxidase B. Safinamide blocks this isoenzyme in a reversible fashion.

Objectives: The aim of this study was to determine monoamine oxidase A (plasma) and B (platelets) enzyme activity in long-term levodopa-treated patients without and with additional oral intake of 50- or 100-mg safinamide or 1-mg rasagiline or first-time intake of rasagiline.

Results: Monoamine oxidase A enzyme activity did not differ between all groups. Patients on rasagiline or safinamide showed lower monoamine oxidase-B enzyme activity compared with patients without monoamine oxidase B inhibitor intake. No impact of the number of previous oral levodopa intakes was found.

Discussion: Rasagiline and safinamide did not essentially differ in terms of inhibition of monoamine oxidase B despite their different pharmacology regarding reversibility of monoamine oxidase B inhibition. In view of the observed, considerable heterogeneity of enzyme activities, we suggest to determine activities of monoamine oxidase A and B to reduce the risk for tyramine-induced hypertension and the serotonergic syndrome during chronic therapy with rasagiline or safinamide.

Key Words: monoamine oxidase, safinamide, rasagiline

(*Clin Neuropharm* 2017;00: 00–00)

Monoamine oxidase (MAO; EC 1.4.3.4) is essential for degradation of biogenic amines in neuronal and glial cells of the brain. Inhibition of MAO provides higher and more sustained levels of biogenic amines concentrations in the synaptic cleft. Two MAO enzyme forms exist, the A and B isoforms. Monoamine oxidases A and B differ in the anatomical localization and preference of substrates. Monoamine oxidase A is primarily found in the placenta, gut, and liver and degrades serotonin and noradrenaline. Monoamine oxidase B predominantly appears in the brain, liver, and platelets, and phenylethylamine, methylhistamine, and tryptamine are its primary substrates.¹ Both, MAO-A and MAO-B, metabolize tyramine and dopamine. Inhibition of one MAO isoform activity only is enabled within a certain and rather low concentration

range of the administered compound. The specificity of a certain MAO subtype inhibition vanes with higher and chronic dosing of the applied drug, which results in an unselective blocking of both MAO-A and MAO-B.^{2,3} Reversible and irreversible MAO-B inhibition is a well-accepted and proven therapeutic principle for patients with Parkinson disease (PD). Currently, there are 2 irreversible MAO-B inhibitors, selegiline and rasagiline, available. Safinamide is a further one, but this compound inhibits MAO-B in a reversible fashion.^{4,5} Generally, the concentration of the substrate essentially determines enzyme activity. In case of drug-induced blocking of MAO, the substrate, that is, the biogenic amine dopamine, and the dosing of the MAO inhibitor influence the enzyme activity of MAO.⁶ In other words, in levodopa/dopa-decarboxylase inhibitor (DDI)-treated patients, peripheral dopamine levels and the MAO inhibitor itself may influence the MAO enzyme activity in the periphery.⁷ In this respect, one must consider that inhibition of dopa-decarboxylase with carbidopa or benserazide does not totally prevent the peripheral decarboxylation of levodopa to dopamine.^{7,8} In a previous trial, we described a decline of MAO-A enzyme activity when exposing it to plasma taken from patients with PD, who were on a long-term therapy with irreversible MAO-B inhibitors with and without levodopa/DDI.² However, this trial examined patients with PD without consideration of the number of additional previous oral levodopa/DDI intakes, when blood sampling was performed 4 hours after the intake of the MAO-B inhibitor.⁷ The objective of the present investigation was to measure MAO-A (plasma) and MAO-B (platelets) activity in chronic levodopa-treated patients with PD with and without rasagiline or safinamide therapy and in relation to previous levodopa intake.

METHODS

Subjects

Exclusion criteria were metabolic disturbances, namely, diabetes; abnormal vitamin values (ie, B₆, B₁₂, folic acid); clinical signs of dementia; or any electrophysiological or morphological evidence (cranial computed tomography or magnetic resonance imaging scan) of additional central nervous system pathology exceeding PD. We investigated 7 cohorts of levodopa/DDI-treated patients. Cohort I was rasagiline-treated patients with PD, cohort 2 took safinamide on a regular basis (for characteristics, see Table 1). Patients of cohort II were additionally subgrouped and analyzed according to their intake of the safinamide dosage (safinamide 50 mg in cohort III, safinamide 100 mg in cohort IV). Patients with PD without any previous MAO-B inhibitor intake and oral levodopa intake on the investigation day were in cohort V. Cohort VI included patients who took rasagiline for the first time in their life, and blood sampling was performed approximately 4 hours after rasagiline intake with concomitant levodopa/DDI application.

Assessment of MAO-A and MAO-B Activity

Determination of MAO-A was performed with plasma as described previously² with several modifications. Monoamine

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Supported by an unrestricted grant from the "Verein zur Durchführung Neurowissenschaftlicher Tagungen e.V.," Würzburg, Germany.

Conflicts of Interest and Source of Funding: T.M. served as PI for trials with rasagiline and safinamide and as advisory board member. E.G. and P.R. have no conflicts of interest.

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TABLE 1. Clinical Characteristics of Cohorts

	Women/Men	Age, y	UPDRS I	UPDRS II	UPDRS III	UPDRS IV	HYS	Interval	LD Intakes	LD Dosage	EN
RAS	4/10	64.08 ± 3.08	4.85 ± 0.52	14.46 ± 0.85	27.15 ± 1.75	4.85 ± 0.52	3.08 ± 0.14	9–10	1.15 ± 0.10	413.4 ± 65.3	925.2 ± 44.14
SAF	9/9	61.94 ± 2.72	5.67 ± 0.62	19.39 ± 1.23	32.33 ± 1.64	4.78 ± 0.39	3.17 ± 0.12	9–10	1.33 ± 0.11	419.6 ± 47.4	923 ± 43.96
SAF 50	2/5	65.43 ± 5.09	6.857 ± 1.01	20.71 ± 2.21	36.43 ± 1.82	5 ± 0.53	3.29 ± 0.18	9–10	1.43 ± 0.2	421.2 ± 45.3	925.4 ± 44.16
SAF 100	7/4	59.73 ± 3.07	4.909 ± 0.732	18.55 ± 1.48	29.73 ± 2.12	4.636 ± 0.56	3.09 ± 0.16	9–10	1.27 ± 0.14	425.4 ± 43.2	915.4 ± 43.25
No MAO-B	3/11	72.83 ± 1.44	5.42 ± 1.07	16.75 ± 1.80	25.25 ± 2.48	3.5 ± 0.26	3.33 ± 0.22	8–9	—	433.3 ± 54.04	933.3 ± 42.16
1st RAS	3/10	72.45 ± 1.52	5.55 ± 1.16	16.64 ± 1.97	25.18 ± 2.72	3.364 ± 0.24	3.36 ± 0.24	12.30–13.30	2.25 ± 0.25	418.2 ± 56.82	920 ± 48.99
ANOVA		s	NS	NS	NS	NS	NS	s	s	NS	NS

All data are given as mean ± SEM.

EN indicates daily entacapone dosage; HYS, score of the Hoehn and Yahr scale; Interval, interval of blood sampling in the morning; LD, daily levodopa dosage; LD intake, number of levodopa intakes on the investigation day before blood sampling; no MAO-B-I, no previous MAO-B inhibitor intake and oral levodopa intake; NS, not significant according to ANOVA between all cohorts in the corresponding column (detailed results not shown); RAS, daily 1-mg rasagiline; s, significant according to ANOVA in the corresponding column (detailed results, ie, of the post hoc analysis, not shown); controls were not included in the analysis in case of missing data); SAF, daily safinamide; SAF 50, daily 50-mg safinamide; SAF 100, daily safinamide 100 mg; UPDRS I–IV, Unified Parkinson's disease Rating Scale parts I to IV; 1st RAS, rasagiline for the first time in their life and blood sampling was performed approximately 4 hours after rasagiline application with concomitant levodopa/DDI application.

oxidase B assessment was performed in platelets.⁹ Total protein in plasma and platelets was assessed using the Bradford assay. Both MAO-A and MAO-B activity assay was conducted using the MAO-Glo Assay (Promega, Germany). For the MAO-A activity assay, the recombinant human MAO-A enzyme was used (Sigma-Aldrich). Luciferase was detected in black 96-well plates (Thermo-Fisher Scientific, Switzerland) on the Mithra² LB 943 Multimode Reader (Berthold Technologies, Germany).

Design

According to Table 1, blood sampling was performed before levodopa intake (ie, cohort V) or after levodopa intake (ie, cohorts I, II, III, IV, and VI). The additional concomitant drug therapy was not changed. We did not aim to evaluate the motor response on the additional application of the MAO-B inhibitor.

Statistics

Values of all groups passed the Shapiro-Wilk normality test. ANOVA was used for the comparisons. The Tukey multiple comparison test was used for the post hoc analysis. *P* value of 0.05 was regarded as significant. Because (1) we split patients of cohort II into cohorts III and IV and (2) gastrointestinal levodopa absorption and peripheral turnover of levodopa to dopamine by dopa decarboxylase are heterogeneous,^{8,10,11} we did not perform a covariate analysis in this descriptive, explorative analysis of this pilot investigation.

Ethics

All subjects gave written informed consent. An independent local institutional review board approved the additional blood collection. This investigation was advertised according § 4 Abs. 23 Satz 3 AMG at the medical association. It was characterized as noninterventional and thus observational because blood sampling is part of the routine surveillance in the treatment for patients with PD.

RESULTS

MAO-A Activity

There were no significant differences between all cohorts ($F_{5,71} = 1.07$, $P = 0.38$) (Fig. 1).

MAO-B Activity

MAO-B activity ($F_{5,71} = 20.25$, $P < 0.001$) varied between groups. The post hoc analysis showed that there is no difference between safinamide and rasagiline in terms of MAO-B inhibition (Fig. 2). There was a significant difference between rasagiline long-term treated patients and the ones without previous MAO-B inhibitor intake and patients 4 hours after first-time intake of rasagiline (Fig. 2). Similar outcomes were obtained in terms of safinamide; again, significant differences of MAO-B activity appeared when compared with the values of patients without previous MAO-B inhibitor intake and patients 4 hours after first-time intake of rasagiline (Fig. 2). Application of safinamide during long-term use did not result in a more pronounced inhibition of MAO-B (Fig. 2). Both relative small safinamide dosing subgroups had a lower MAO-B activity than patients with PD without MAO-B inhibitor intake (Fig. 2), but the 50-mg safinamide dose did not differ from patients with first-time rasagiline intake in contrast to safinamide 100-mg group (Fig. 2). No significant difference was found between patients without previous levodopa and MAO-B inhibitor intake and

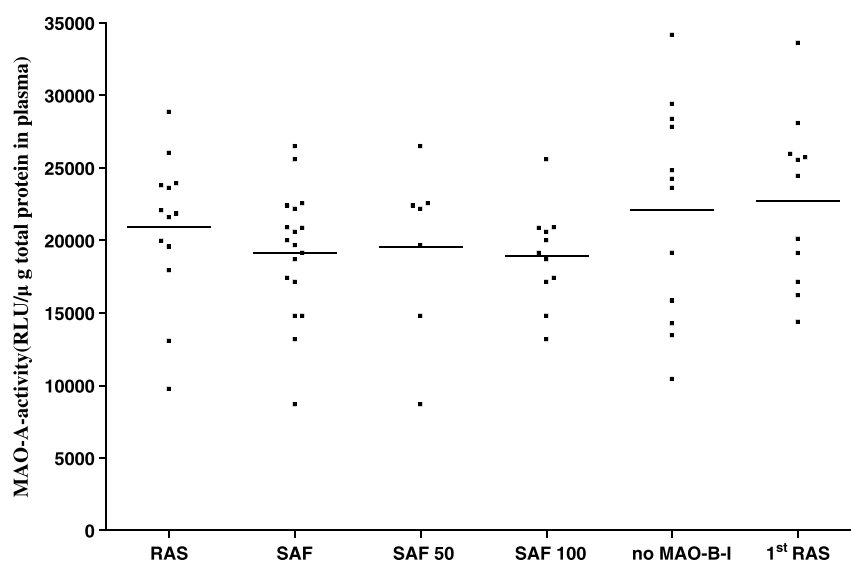


FIGURE 1. Scatterplot of MAO-A activity in the various cohorts. All cohorts showed lower enzyme values than the controls ($P < 0.001$ according to the post hoc analysis). Cohorts: RAS, daily 1-mg rasagiline; SAF, daily safinamide; SAF 50, daily 50-mg safinamide; SAF 100, daily safinamide 100 mg; no MAO-B-I, no previous MAO-B inhibitor intake and oral levodopa intake; 1st RAS, rasagiline for the first time in their life and blood sampling was performed approximately 4 hours after rasagiline application with concomitant levodopa/DDI application. RLU indicates relative light unit.

patients with previous levodopa and first-time MAO-B inhibitor intake (Fig. 2).

DISCUSSION

We show that both, rasagiline and safinamide, does not affect “ex vivo” determined MAO-A enzyme activity. We found no impact of the number of levodopa/DDI intakes on the investigation day on MAO-A activity.⁷ In view of our present results, we assume that the previous hypothesis of MAO-A inhibition by rasagiline,

respectively selegiline, during long-term application according to the outcomes of an earlier trial warrants further experiments particularly in healthy and age-matched controls. In that earlier investigation, we indirectly measured MAO-A enzyme activity in a similar fashion as in this study and found a reduced MAO-A activity in comparison with the controls.² To date, we cannot exclude in both trials that the applied MAO activity influencing drugs was not fully metabolized and excreted, when the plasma sample was taken.⁷ Thus, during chronic application, accumulation of the compound may also exhibit a certain effect on MAO-A in the

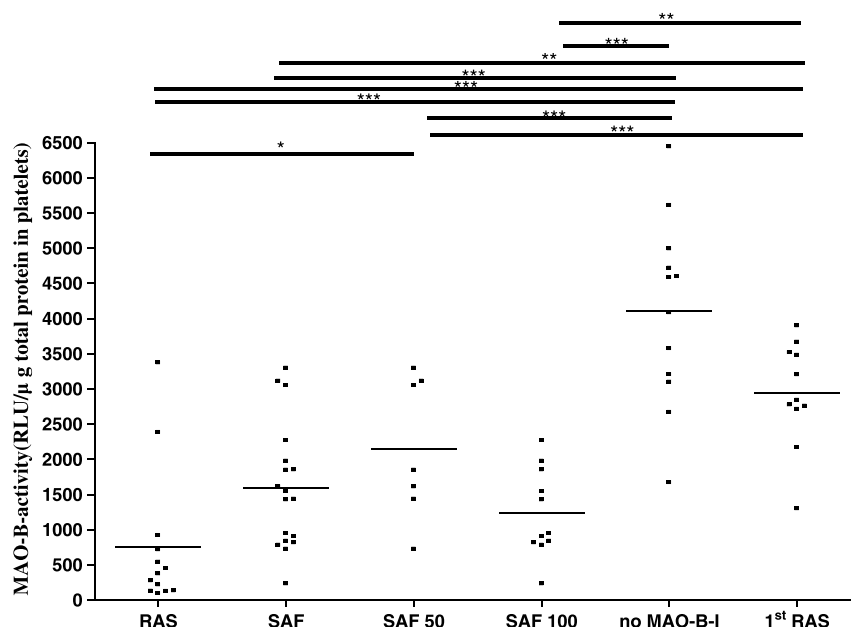


FIGURE 2. Scatterplot of MAO-B activity in the various cohorts. Cohorts: RAS, daily 1-mg rasagiline; SAF, daily safinamide; SAF 50, daily 50-mg safinamide; SAF 100, daily safinamide 100 mg; no MAO-B-I, no previous MAO-B inhibitor intake and oral levodopa intake; 1st RAS, rasagiline for the first time in their life and blood sampling was performed approximately 4 hours after rasagiline application with concomitant levodopa/DDI application. RLU indicates relative light unit.

applied in vitro experiment for the indirect measurement of MAO-A.^{2,9} Another hypothesis would be that elevation of peripheral dopamine generation by levodopa application and synthesis of other biogenic amines resulting from chronic DDI administration may also generate an effect on MAO enzyme activity in general.¹² This could contribute to the observed scattering of MAO-A and MAO-B enzyme activity in all cohorts. A further complementing reason may particularly be an elevated generation of dopamine, which is an MAO substrate and thus inhibits MAO enzyme activity. The concomitant intake of levodopa with a DDI does not prevent that a considerable amount of levodopa still undergoes peripheral decarboxylation to dopamine.¹² Thus, the individual variability of levodopa plasma bioavailability after gastrointestinal levodopa absorption may also contribute to the observed variation of peripheral MAO enzyme activity.¹¹ Here, we found no impact of previous levodopa intake on MAO-A enzyme activity and cannot postulate such an effect in the case of MAO-B because of the missing control group in contrast to our earlier trial.² A further theoretical cause may be that this phenomenon additionally at least partially results from the presence of endogenous MAO inhibitors.¹³

Rasagiline and safinamide are both MAO-B inhibitors. Both drugs in the applied dosages do not essentially differ in reducing of MAO-B activity, despite their different pharmacological properties in terms of reversibility of MAO-B blocking. The more pronounced reduction of MAO-B activity after repeated MAO-B inhibitor intake compared with first-time administration of rasagiline may hypothetically result from a certain cumulative effect generated by the MAO-B inhibitor on MAO-B enzyme activity or on MAO-B synthesis. There was no difference of MAO-B activity between cohorts III and VI, but the number of patients on a 50-mg safinamide regimen is rather low. Therefore, we refrain from claiming that the effects of chronic 50-mg safinamide intake on MAO-B enzyme activity correspond to the one of first-time dosing of 1-mg rasagiline.

We demonstrate heterogeneity of the extent of MAO inhibition by rasagiline and safinamide in Figures 1 and 2. Generally, there is a discussion on the so-called “cheese” effect during MAO inhibition. In view of the observed peripheral variability of MAO inhibition, we suggest that only individuals with a pronounced MAO inhibition are at risk to develop this tyramine-induced hypertension during MAO inhibition. Therefore, assessment of MAO-A and MAO-B activity during MAO inhibition may hypothetically help to identify these “cheese effect at risk” individuals and allow to recommend them preventive dietary restrictions. We also recommend these assessments particularly during combination of serotonin reuptake inhibitors with MAO-B inhibitors. This may reduce the risk for onset of a serotonergic syndrome. Moreover, the observed heterogeneous enzyme activity suggests that fixed dosing of MAO-B inhibitors should not be recommended in clinical practice. We suggest that it would be better to titrate and apply MAO-B inhibitors in various dosages to achieve the optimum motor response in relation to levodopa dosing. In this regard, assessment of enzyme activity may be advantageous.

Limitations of this present pilot investigation are the relatively small number of subjects in some cohorts; the absent comparison with healthy, age-matched controls; the missing determination of peripheral levodopa and dopamine concentrations; and the heterogeneity of levodopa dosing—respective moments of levodopa dosing. Therefore, these outcomes should be regarded as preliminary.

Nevertheless, these results provide some deeper insight in the complexity of drug-induced regulation of enzyme activity in chronic treated patients with PD. This kind of research warrants further studies to investigate the effects of short- and long-term levodopa treatment on MAO inhibition. To date, certain long-term effects, such as induction of enzyme generation, during chronic levodopa/DDI supplementation are not well investigated.

In conclusion, we show with this observational pilot investigation that rasagiline and safinamide presumably inhibit MAO-B but not MAO-A.

ACKNOWLEDGMENT

The authors thank the patients and Gabi Öhm, Kathrin Eilert, Marion Marg, Hartmut Rothe, Matthias Kuchler, Sven Lütge, and Thomas Haas. They are also grateful to Professor Dr Manfred Gerlach and Thomas Elpel, Center of Mental Health, University Hospital Würzburg, Germany, for the subfractioning of blood samples.

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