BNL-107759-2015-JA

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Submitted to Neuropsychopharmacology

July 2014

Biological, Environmental and Climate Sciences Department
Brookhaven National Laboratory

U.S. Department of Energy
Office of Science
Office of Biological and Environmental Research

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Evidence that formulations of the selective MAO-B inhibitor, selegiline, which bypass first-pass metabolism, also inhibit MAO-A in the human brain

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count:

abstract (252)
text (3374)
number of references: 43
number of figures: 2
number of tables: 3
key words: brain MAO-A; MAO inhibitors; PET; selegiline; Zydis selegiline; Emsam, selegiline transdermal system; buccal mucosa absorption

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ABSTRACT

Selegiline is a selective, irreversible inhibitor of monoamine oxidase B (MAO-B) at the conventional dose (10 mg/day oral) that is used in the treatment of Parkinson's disease. However, controlled studies have demonstrated antidepressant activity for high doses of oral selegiline and for transdermal selegiline suggesting that when plasma levels of selegiline are elevated, brain MAO-A might also be inhibited. Zydis selegiline (Zelapar®) is an orally disintegrating formulation of selegiline, which is absorbed through the buccal mucosa producing higher plasma levels of selegiline and reduced amphetamine metabolites compared to equal doses of conventional selegiline. Although there is indirect evidence that Zydis selegiline at high doses loses its selectivity for MAO-B, there is no direct evidence that it also inhibits brain MAO-A in humans. We measured brain MAO-A in 18 healthy men after a 28-day treatment with Zydis selegiline (2.5, 5.0 or 10 mg/day) and in 3 subjects receiving the selegiline transdermal system (Emsam patch, 6 mg/day) using PET and the MAO-A radiotracer [^{11}C]clorgyline. We also measured dopamine transporter (DAT) availability in three subjects from the 10 mg group. The 10 mg Zydis selegiline dose significantly inhibited MAO-A (36.9 ± 19.7%, range 11-70%, $p<0.007$)) but not DAT; and while Emsam also inhibited MAO-A (33.2 ± 28.9 (range 9-68%) the difference did not reach significance ($p=0.10$) presumably because of the small sample size. Our results provide the first direct evidence of brain MAO-A inhibition in humans by formulations of selegiline, which are currently postulated but not verified to target brain MAO-A in addition to MAO-B.

INTRODUCTION

Developed in the 1950s, monoamine oxidase inhibitors (MAOIs) were the first effective medications for the treatment of depression, and probably remain some of the most effective agents today despite the introduction of newer drugs (Shulman et al., 2013). Unfortunately, the early MAOIs were non-selective and irreversibly inhibited both MAO isoforms (MAO-A and MAO-B) in the gut as well as the brain, causing serious hypertensive reactions following the ingestion of foodstuffs containing tyramine. This is referred to as the "cheese effect" (Finberg and Gilman, 2011). The antidepressant effect of MAO inhibitors is generally attributed to their inhibition of brain MAO-A, which breaks down the neurotransmitters serotonin and norepinephrine, that are involved with mood and which are targets for most antidepressants (Lipper et al., 1979; Finberg, 2014, review).

Selegiline is an irreversible inhibitor of MAO-B with selectivity for MAO-B vs MAO-A (Robinson, 1985; Santana et al., 2008). At low doses (10 mg/day, oral) selegiline specifically inhibits brain MAO B while leaving brain MAO-A substantially intact (Riederer and Youdim, 1986; Fowler et al., 2001), and is used to treat Parkinson's disease (PD). However, controlled studies have demonstrated antidepressant activity for high doses of oral selegiline, suggesting that when plasma levels of selegiline are elevated, its selectivity for MAO-B is lost resulting in MAO-A inhibition, producing antidepressant activity and requiring the limitation of dietary tyramine (Prasad et al., 1988; Schulz et al., 1989; Sunderland et al., 1985, 1994, Mann et al., 1989).

To reduce the inhibition of gut MAO-A, two different formulations of selegiline have been developed. Selegiline transdermal system (Emsam) enables absorption from

the skin directly into the bloodstream. Preclinical measurements in guinea pigs showed preferential inhibition of CNS MAO-A and MAO-B compared with intestinal and hepatic tissues unlike oral administration, which resulted in similar levels of brain and gut MAO-A inhibition (Mawhinney et al, 2003). Pharmacokinetic studies in healthy humans comparing the selegiline transdermal system with oral selegiline capsules showed a significant reduction in both tyramine pressor sensitivity and amphetamine metabolites (Azzaro et al, 2006, 2007). The use of selegiline transdermal system enabled controlled clinical trials to treat depression without dietary restrictions (Bodkin and Amsterdam, 2002; Amsterdam, 2003; Feiger et al, 2006).

Zydis selegiline (Zelapar®) is a freeze-dried formulation which when allowed to dissolve on the tongue without swallowing, is absorbed primarily through the buccal mucosa into the bloodstream thereby reducing the opportunity for first pass metabolism and inhibition of gut MAO. Pharmacokinetic studies have shown that Zydis selegiline inhibits MAO-B at 1/8 of the conventional oral dose (10 mg/day) and decreases amphetamine metabolites by 80-90% (Clarke et al., 2003a,b; Lohle and Storch, 2008). In addition, repeated doses of Zydis selegiline 10 mg (but not the 1.25 mg dose) resulted in a significant reduction in the urinary excretion of 5-hydroxy-indoleacetic acid (5-HIAA), a peripheral marker for MAO-A activity consistent with inhibition of MAO-A in addition to MAO-B, with repeated 10 mg dosing (Clark et al., 2003a). However, even though the antidepressant activity of high dose and other formulations of selegiline have been attributed to the inhibition of brain MAO-A (Finberg, 2014 for review), this has never been confirmed in humans.

The purpose of this study was to investigate whether formulations of selegiline which bypass first-pass metabolism also inhibit brain MAO-A in humans utilizing positron emission tomography (PET) imaging and the MAO-A radiotracer [^{11}C]clorgyline (Fowler et al., 1996). We examined different daily doses of Zydis selegiline (2.5, 5.0 and 10 mg) given for 28 days to 18 healthy human subjects and by the selegiline transdermal system (6 mg/day) in 3 subjects. Since dopamine transporter (DAT) inhibition has also been suggested as a possible mechanism for the antidepressant activity of selegiline (Mann et al, 1989), we also measured DAT availability in 3 subjects receiving the 10 mg dose of Zydis selegiline using the DAT radiotracer, [^{11}C]cocaine (Fowler et al., 1989).

MATERIALS AND METHODS

This study was carried out at Brookhaven National Laboratory and approved by the local Institutional Review Board (Committee on Research Involving Human Subjects, Stony Brook University). All subjects gave their written informed consent.

Subjects and study design: Twenty eight subjects were screened and twenty normal healthy non-smoking males age 18-38 years were enrolled. Inclusion criteria were: healthy, non-smoking males, body mass index (BMI, kg/m^2) $\geq$ 21 and $\leq$ 30, able to understand and give informed consent. Key exclusion criteria included presence of a significant acute or chronic medical disorder that might complicate or interfere with MAO inhibitor therapy, current or past psychiatric or neurological disease, cardiovascular disease, endocrinological disease, history or presence of phenylketonuria, and history of substance abuse, including alcohol or nicotine abuse as defined by DSM-IV criteria,

within the past 12 months. All subjects were instructed to abstain from taking prescription or nonprescription drugs within 7 days (2 months for neuroleptics and fluoxetine or any other drug with a prolonged half-life) and to adhere to a low tyramine diet during the study and 2 weeks following study participation. Subject demographics are summarized in Table 1. All study participants also consented to participate in an imaging genetic study and provided blood sample for MAO-A genotyping as described previously (Sabol et al., 1998).

This was an open-label, non-randomized, single-center study designed to evaluate the effects of administration of 3 different daily doses of Zydis selegiline (2.5 mg, 5.0 mg, and 10.0 mg) on healthy subjects for 28 days on brain MAO-A. Six subjects were assigned to each dose. Three subjects were assigned to the Emsam group (6 mg/24 hr). One subject participated in both the Zydis selegiline (2.5 mg) and the Emsam groups. Zelapar Orally Disintegrating Tablets®, each containing 1.25 mg of selegiline Zydis formulation, were administered orally by dissolution on the tongue (not swallowed). Instructions for dosing and the first dose of Zydis selegiline was observed by study personnel after the baseline PET scan. Subjects were instructed not to take the last tablets on the day before the second PET scan but to wait since it would be administered 2 hrs before the last PET scan. Each 6 mg/24 hr Emsam patch was supplied as a 20 mg/20 cm^2 transdermal system and the first patch was applied following the baseline PET scan. The duration of treatment was 28 days and the last patch was applied 2 hours prior to the second PET scan.

A treatment log was provided to each subject and weekly phone reminders were made to take the medication and to follow dietary restrictions. Missed doses were noted

on the dispensing logs. Subjects who missed 20% of the doses were withdrawn from the study. Subjects were instructed to return unused study drugs and empty wrappers, which were inventoried to assess subject compliance throughout the treatment period.

Zydis selegiline tolerability was assessed as oropharyngeal irritation at baseline and Day 28. Blood and urine samples for clinical laboratory tests were obtained at screening, day 28 and the post-treatment follow-up visit. Adverse events queries were conducted at each visit and during periodic telephone calls during dosing. Vital signs were measured at screening, baseline, immediately before and after each PET scan, and at the follow-up visit.

[^{11}C]Clorgyline and [^{11}C]cocaine PET scans: [^{11}C]Clorgyline synthesis and PET scans were carried out as described previously (MacGregor et al. 1988; Ali-Klein et al., 2008). Each subject had 2 PET scans with [^{11}C]clorgyline (average 6.6 ± 0.9 mCi/scan; specific activity, 0.9 ± 0.4 mCi/nmol at time of injection), one at baseline and the second at 28 days after dosing. An arterial plasma input function for [^{11}C]clorgyline was measured (Alexoff et al., 1995). Three subjects who received the 10 mg Zydis selegiline dose also had a scan with [^{11}C]cocaine (average 7.2 ± 0.4 mCi/scan; specific activity, 0.95 ± 0.31 mCi/nmol at time of injection) on the same day (two hours apart) using the synthesis and scanning protocol reported previously (Volkow et al., 1997).

Image analysis: Emission data were attenuation corrected and reconstructed using filtered back projection. ROI placement for the [^{11}C]clorgyline and the [^{11}C]cocaine scans was made as described previously (Fowler et al., 2010; Volkow et al., 2009).

Composite images were created by first normalizing each subject's scan to a standard template. Images from each of the six MAO-A subjects or three DAT subjects were then averaged at baseline and post dosing with 10 mg of Zydis selegiline.

Data analysis: For quantification of MAO-A activity, PET time-activity data for [^{11}C]clorgyline from different brain regions and time-activity data in arterial plasma were used to calculate the model terms K_1 and λk_3 at baseline and after Zydis selegiline and Emsam. The model term K_1 is related to blood flow (F) and permeability surface area product (PS) via the $K_1 = F(1-e-(PS/F)$. The model term λk_3 is a function of the concentration of catalytically active MAO-A molecules where k_3 is related to the irreversible binding of [^{11}C]clorgyline by MAO-A, k_2 is related to the efflux of radiotracer from brain to blood and λ is defined as K_1/k_2 and is independent of blood flow (Fowler et al., 1996; Logan et al., 1991). [^{11}C]Clorgyline binding (λk_3) in human brain has recently been shown to be proportional to MAO-A protein content (Tong et al., 2013). For the [^{11}C]cocaine scans, PET time-activity curves in the caudate, putamen and cerebellum and the arterial input function were used to calculate K_1 and the non-displaceable binding potential (BP_{ND}) as an index of DAT availability using the Logan plot (Logan et al., 1990).

Estimation of the degree of inhibition of [^{11}C]clorgyline binding to MAO-A by Zydis selegiline and Emsam. For each subject, a λk_3 value for each of the 12 ROI's was determined at baseline and after Zydis selegiline or Emsam administration. A composite global λk_3 value for each subject at baseline and after drug was also determined by

averaging the regional λk_3 values. For each subject, the percent inhibition of MAO-A was determined from the percent change in λk_3 relative to baseline for each of the ROIs and these values were averaged to give an average percent change (mean±SD) at each drug dose.

Statistical analysis of [^{11}C]clorgyline data and [^{11}C]cocaine data. A one-way ANOVA analysis was used to examine whether there was a significant dose-related change in the % inhibition of [^{11}C]clorgyline binding (λk_3) after a 28 day dosing regimen for the 2.5 vs 5.0 vs 10.0 mg Zydis selegiline doses. Pair-wise differences were examined via the Tukey method at the family-wise error rate of 0.05 (1-tailed) with 2-tailed test being used to examine differences in K_1. Unpaired t test was used to examine whether there was a statistically significant difference in the percent change in [^{11}C]clorgyline binding for the Zydis selegiline 10 mg group and the Emsam group. For the 10 mg group, we subjected the individual ROI data to a repeated measures ANOVA analysis to determine whether the percent change differed between the ROIs. To assess whether subject characteristics (age and BMI) and weight-adjusted dose influenced the percent change in MAO-A we performed correlation analysis for each dose group. A comparison of BP_{ND} for [^{11}C]cocaine at baseline and after the 10 mg dose of Zydis selegiline was made using a paired t-test, 2-tailed. We also performed a correlation analysis between the % change in DAT and the % change in MAO-A for the 3 subjects in whom both measures were made.

RESULTS

There was no statistical difference in age or BMI for the three dose groups for Zydis selegiline (Table 1). All doses of Zydis selegiline and the Emsam patch were tolerated and there were no adverse events and no changes in vital signs after the 28-day period. Weekly phone call reminders and tablet and patch container inventories indicated compliance with the medication instructions.

Effect of orally disintegrating selegiline (Zydis selegiline) and selegline transdermal system (Emsam) on [^{11}C]clorgyline binding in the brain

There were no significant differences in K_1 after either Zydis selegiline or Emsam administration (Table 2). The average values of λk_3 for the 2.5, 5.0 and 10 mg doses of Zydis selegiline and Emsam groups are presented in Table 2. There was no difference between the baseline λk_3values for the three groups, nor were there differences between the baseline and post treatment values of λk_3 for the 2.5 and 5.0 mg groups. However, the 10 mg group showed a significant reduction of λk_3 which corresponded to a 36.9±19.7% inhibition of MAO-A though the percent reduction varied between subjects (range 11-70%). Table 2 also shows an increasing reduction in λk_3 from baseline values as a function of Zydis dose although only the 10 mg dose reaches statistical significance. MAO-A reduction can be seen in the averaged PET images at baseline and after treatment with 10 mg Zydis selegiline (Figure 1, top row). The λk_3 reduction with Emsam ranged from 7.5% to 68% with no significant difference between the 10 mg Zydis selegiline and the Emsam group. We also compared percent change in λk_3 for the twelve individual regions of interest (ROI's) for the 10 mg group and found a significant region effect

(Repeated ANOVA in 12 ROI, F=1.2, p=0.04) driven by lower inhibition in the cerebellum relative to other regions of interest.

Individual values for the composite λk_3 values at baseline and after treatment as well as a plot of percent change in λk_s for each of the subjects are shown in Figure 2. There were no significant associations between percent change in λk_3 after Zydis selegiline and subject's age, BMI, genotype or weight-adjusted dose of drug. However, for the 10 mg Zydis selegiline group there was a significant correlation between baseline λk_3 and percent change in λk_3 with higher baseline λk_3 values showing the largest decreases in MAO-A after drug (R=0.83; p=0.04)

Baseline λk_3*'s* differed by 4% for the one subject (#094530) who participated in both the Zydis selegiline group and the Emsam group 14 months apart. Test/retest reproducibility for λk_3 was 10.9±5.0% from prior human studies (Fowler et al., 1996). Thus, our results indicate that percent MAO-A inhibition with the 10 mg Zydis selegiline dose is at least 3 fold higher than the test-retest variability.

[^{11}C]Cocaine binding in brain

For three of the subjects in the 10 mg Zydis selegiline group, we measured DAT availability with [^{11}C]cocaine at baseline and after treatment. The average non displaceable binding potential (BP_{ND}) though lower with Zydis selegiline did not differ significantly from the baseline (Figure 1, bottom row; Table 3). There was a significant decrease in the value of K_1 after Zydis selegiline in caudate and putamen but the decrease in cerebellum was not significant (Table 3).

DISCUSSION

There is growing evidence that different formulations and different routes of administration of the same drug molecule can have a profound effect on drug

bioavailability, pharmacokinetics and pharmacodynamics. (Smith and Uhl, 2009). We report here the first measurements confirming brain MAO-A inhibition in humans receiving formulations of selegiline, which reduce or bypass first pass metabolism. We found that 10 mg of Zydis selegiline for 28 days loses its selectivity for MAO-B and significantly inhibits brain MAO-A and that the Emsam patch for 28 days showed >30% inhibition of MAO-A in two of the three subjects tested.

Our results confirming inhibition of brain MAO-A with high dose Zydis selegiline and with the Emsam patch provide the first direct evidence in the humans that brain MAO-A inhibition may contribute to its pharmacological and therapeutic profile, particularly for depression. They are consistent with those of Clarke et al. who reported that repeated doses of high dose Zydis selegiline reduces the urinary excretion of 5-HIAA, a peripheral marker for MAO-A activity (Clark et al., 2003) and with preclinical studies with the selegiline transdermal system which showed targeted inhibition of the MAO enzymes in the CNS while limiting MAO-A inhibition in the gut (Mawhinney et al, 2003).

Meyer *et al* (2006) reported that patients with major depressive disorder have an average 34% elevation of brain MAO-A, which may contribute to the monoamine imbalance in depression. We can speculate that brain MAO-A inhibition by formulations of selegiline which result in brain MAO-A inhibition, may contribute to a rebalancing of brain monoamines. Interestingly, there is a high comorbidity between smoking and depression (Glassman et al, 1990). Biologically based self-medication hypotheses have been proposed to account for this observation including nicotine-induced release of norepinephrine and dopamine which are neurotransmitters mediating arousal and stimulation (Lerman et al., 1996; Pomerlau and Pomerlau, 1984). Since tobacco smoke inhibits brain MAO-A by ~30 % (Fowler *et al*, 1996),

brain MAO-A inhibition by cigarette smoke may also contribute to a rebalancing of brain monoamines thus alleviating depression symptoms.

Even though the primary pharmacological action of selegiline is MAO-B inhibition, its pharmacology is complex (Finberg, 2014 for review) and other mechanisms including the actions of amphetamine metabolites (Engberg et al, 1991), increased DAT expression (Lamensdorf *et al*, 1999) and DAT upregulation (Wiener et al, 2009) have also been proposed to account for the antidepressant efficacy of high doses of selegiline (Mann *et al*, 1989). Significant decreases in amphetamine metabolites with transdermal (Azzaro et al, 2007) and orally disintegrating formulations of selegiline (Clarke et al, 2003a) reduce the likelihood that selegiline metabolites play a role in the pharmacological effects of these formulations. Similar to our prior PET study with conventional selegiline (10 mg/day for 1 week; Fowler *et al*, 2001), we did not find significant brain DAT inhibition by 10 mg Zydis selegiline. We note that DAT inhibitor drugs like methylphenidate and modafinil at therapeutic doses inhibit >50% of DAT (Volkow et al., 2002; Volkow et al., 2009) whereas the DAT reduction we observed with Zydis selegiline for 3 subjects was <15% which is not statistically significant and is unlikely to contribute to the pharmacological profile of this formulation.

There was a large individual variability in the percent change in [^{11}C]clorgyline binding (λk_3) within the 10 mg Zydis selegiline and the Emsam groups (Figure 2). We found no significant associations between the percent change in [^{11}C]clorgyline binding and subject demographics nor with the weight-adjusted dose of Zydis selegiline. However, there was a significant correlation between baseline λk_3 and percent change in λk_3 for the 10 mg Zydis selegiline group, with higher baseline λk_3 values showing the largest decreases in MAO-A after

drug. However the sample size is small and the mechanism(s) underlying this relationship is unclear.

There is a common polymorphism in the promoter region of the MAOA gene that has two common alleles (4-repeat and 3-repeat) that occur in a 60:40 ratio in male humans which are commonly referred to as high and low MAOA genotypes as defined by their significantly different transcriptional activities (Sabol et al., 1998). The identification of this polymorphism led to the hypothesis that MAOA genotype may serve as a marker of MAOA gene function and to many studies of the association between high and low MAOA genotype and vulnerability to environmental stressors in humans (Dorfman et al., 2014). We include MAOA genotype as one of the variables which characterize our subjects. In a prior study we found that high and low MAOA genotype does not correlate with [^{11}C]clorgyline binding in brain in healthy male volunteers (Fowler et al., 2007). In this study, we found no association with the percent change in MAO-A activity with the high dose Zydis selegiline and Emsam groups and brain MAO-A levels as measured with [^{11}C]clorgyline.

Our study had some limitations that warrant further investigation. This includes the small sample size particularly for the Emsam measures and the DAT measures. In addition, this was an outpatient study. Subjects were contacted weekly to remind them to take the medication as instructed and each subject returned at least 80% of their empty foil wrappers indicating to us that most of the medication was taken. Selegiline plasma measures would have allowed us to assess if plasma levels of selegiline correlated with the level of brain MAO-A blockade and future studies in a larger number of subjects would enable such a correlation to be explored.

In summary, this is the first direct confirmation of brain MAO-A inhibition in humans by high dose Zydis selegiline and the Emsam selegiline both of which are currently postulated but

not verified to inhibit brain MAO-A in addition to MAO-B. This suggests that brain MAO-A inhibition may account for some of the pharmacological and therapeutic effects of high dose Zydis selegiline and other formulations of selegiline, which avoid first pass metabolism, including reports of antidepressant activity.

ACKNOWLEDGMENTS

This study was carried out using the infrastructure of Brookhaven National Laboratory under contract DE-AC02-98CH10886 and was funded by Valeant Pharmaceuticals International. We thank Michael Schueller for cyclotron operations and Donald Warner for PET operations. N.D.V, was supported by the National Institute on Drug Abuse and M.J., G.J.W., F. T and K.A-T. were supported by the National Institute of Alcohol Abuse and Alcoholism; J.S.F. was supported in part from a K award from NIH (K05DA020001); F.M-P was employed by Valeant Pharmaceuticals International at the time of this study and joined Targeted Medical Pharma Inc after this study was completed. M. G. was an employee of Valeant Pharmceuticals International at the time of the study. This work was presented in part at the 63rd Annual Meeting of the American Academy of Neurology, Honolulu Hawaii, April 13, 2011 and the International Congress of Parkinson's Disease and Movement Disorders meeting in Toronto June 8, 2011

AUTHOR CONTRIBUTIONS

J.S.F., S.F. and N.D.V. wrote the manuscript. J.S.F., S.F., F.M-P, G.-J.W. and M.G. designed the study. F.T, J.S.F., G-J.W., M.J., D.L.A., K.A-T., P.C., C.S., Y.X., L.M., E.S. performed the work. J.L. and J. S. F. analyzed the data.

DISCLOSURES

This study was funded by Valeant Pharmaceuticals International. The authors disclose no other conflicts of interest that might potentially bias this work.

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Table 1. Demographic characteristics of study participants. Abbreviations Hi: high MAOA genotype; Lo: low MAOA genotype

	Zydis selegiline (2.5 mg)	**Zydis selegiline (5.0 mg)**	**Zydis selegiline (10 mg)**	**Emsam (6 mg)**
N	6	6	6	3
Age (yrs)	27±5	29±7	26±5	32±2
BMI (kg/m^2)	24.8±2.0	25.6±2.9	27.8±2.5	26.3±2.0
Genotype	4Hi/2Lo	3Hi/3Lo	3Hi/3Lo	2Hi/1Lo

Table 2. Model terms for [^{11}C]clorgyline binding at baseline and after treatment with different doses of Zydis selegiline and the single dose of Emsam.

	K_1	**% change**	**P value**	λk_3	**% change**	**P value**
Zydis (2.5 mg)						
baseline	0.39±0.04			0.30±0.02		
drug	0.43±0.06	9.3±14.3	0.16	0.29±0.04	2.1±13.5	0.3
Zydis (5.0 mg)						
baseline	0.40±0.16			0.32±0.04		
drug	0.40±0.07	2.8±18.3	0.8	0.29±0.04	7.4±16.4	0.13
Zydis (10 mg)						
baseline	0.40±0.05			0.32±0.04		

drug	0.37±0.03	-5.1±14.2	0.4	0.19±0.05	36.9 ± 19.7	0.007
Emsam 6 mg)						
baseline	0.37±0.08			0.34±0.04		
drug	0.34±0.08	-4.7±23.4	0.7	0.22±0.09	33.2 ± 28.9	0.10

Table 3. Model terms for [^{11}C]cocaine binding at baseline and after Zydis selegiline

Region	**(Baseline)**	**(Zydis selegiline)**	**P value**
K_1			
caudate	0.47±0.07	0.40±0.08	0.01
putamen	0.53±0.07	0.43±0.06	0.005
cerebellum	0.47±0.10	0.37±0.03	0.16
BP_{ND}			
Caudate	0.75±0.18	0.63±0.25	0.2
Putamen	0.90±0.15	0.83±0.21	0.2

Figure Legends

Figure 1. Composite PET images of MAO-A (λk_3, top row) and of DAT ($BP_{ND,}$ bottom row) at baseline and after 10 mg of Zydis selegiline administration.

Figure 2. Values of λk_3 for each individual subject at baseline and after a 28 day Zydis selegiline treatment for the 2.5 (upper left), 5.0 (upper right) and 10 mg (lower left) groups. Average values for the three Emsam treated subjects are also shown.

Figure 1

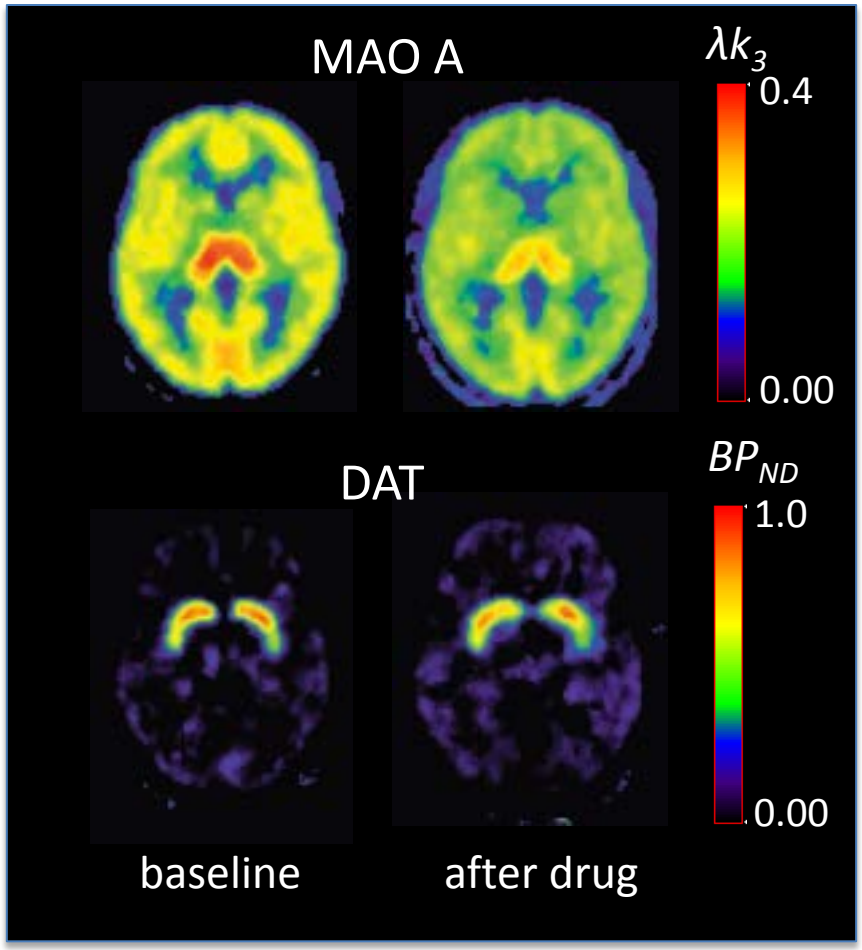
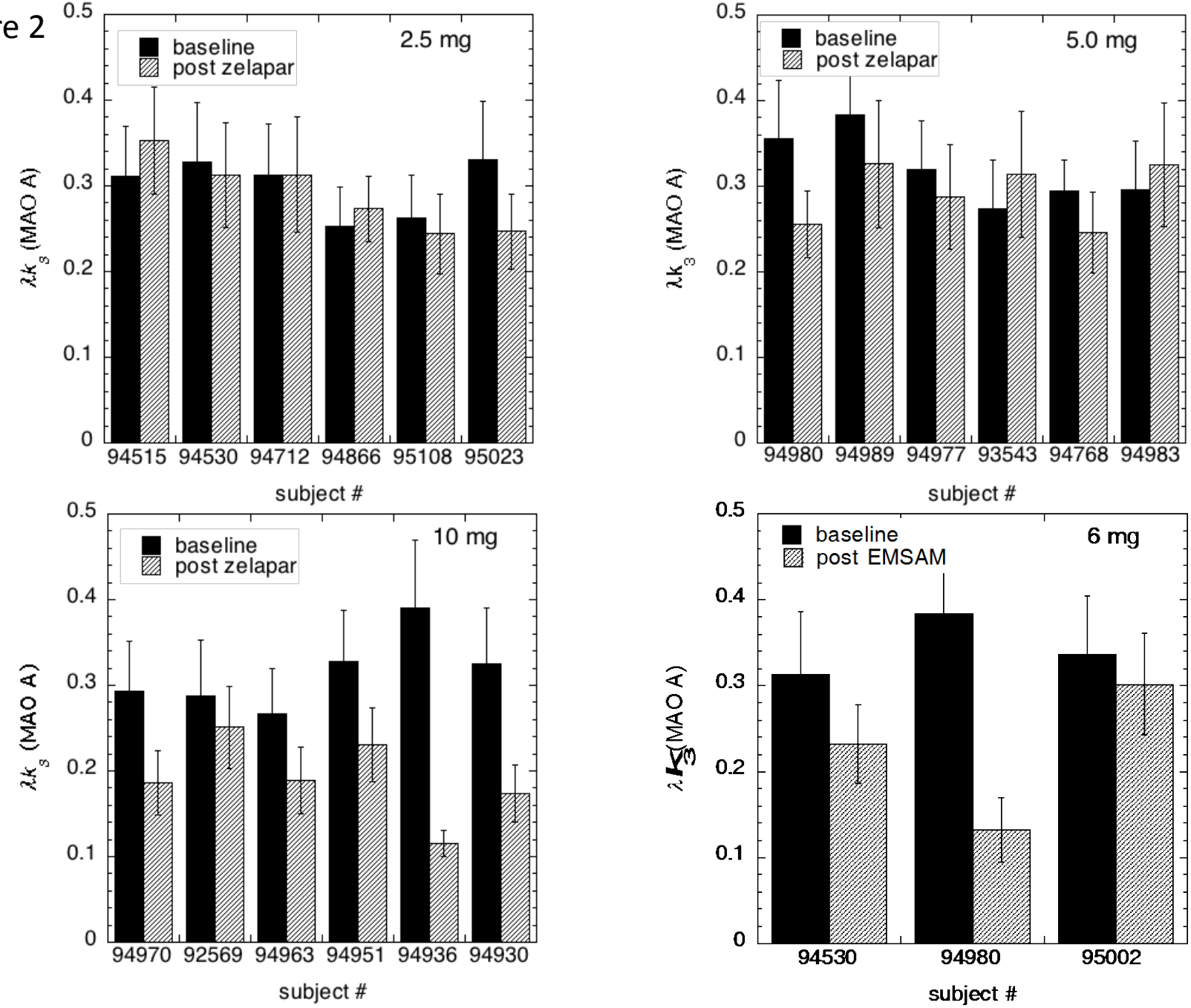


Figure 2


baseline
post zelapar
2.5 mg
λk_3 (MAO A)
0.5
0.4
0.3
0.2
0.1
0
94515 94530 94712 94866 95108 95023
subject #
baseline
post zelapar
5.0 mg
λk_3 (MAO A)
94980 94989 94977 93543 94768 94983
subject #
baseline
post zelapar
10 mg
λk_3 (MAO A)
94970 92569 94963 94951 94936 94930
subject #
baseline
post EMSAM
6 mg
λk_3 (MAO A)
94530 94980 95002
subject #

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