Absorption and Presystemic Metabolism of Selegiline Hydrochloride at Different Regions in the Gastrointestinal Tract in Healthy Males

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Purpose. The absorption and disposition of selegiline (SEL) and its metabolites N-desmethylselegiline (DMS), L-methamphetamine (MET), and L-amphetamine (AMP) were assessed in 8 healthy male volunteers at proximal and distal regions of the intestine relative to oral administration (in the stomach) to determine if intestinal site dependence contributed to the erratic oral absorption of selegiline hydrochloride which is manifest as low and variable bioavailability.

Methods. An open-label, four-way crossover, single dose pharmacokinetic study comparing the bioavailability of 10 mg selegiline hydrochloride administered to healthy young males as a solution by the oral route (in the stomach) and by a nasoenteric tube to the following three sites: duodenum, jejunum and terminal ileum was conducted. Infusions were administered over a 1 minute interval and a two week washout was observed between treatments. Samples were taken over 96 hours and analyzed by LC/MS/MS.

Results. Selegiline exposure was greatest following administration to the stomach (~150% > duodenum or jejunum) and least in the terminal ileum (~33% less than duodenum or jejunum). Duodenal and jejunal sites were equivocal based on selegiline absorption and subsequent metabolism. While both AMP and MET exposure was equivalent at all dosing sites, DMS exposure was less (~18%) at the terminal ileum.

Conclusions. The oral absorption of selegiline is neither permeability-limited or intestinal site-dependent. Stomach absorption may bypass presystemic metabolism. The reduced DMS exposure at the terminal ileum is consistent with the theorized presystemic formation of DMS via luminal P450 enzymes and the density of these enzymes in the duodenum and jejunum relative to the ileum. AMP and MET metabolites were insensitive to dosing site consistent with their hepatic formation. The true magnitude of these effects would require multiple dosing as single dose pharmacokinetics do not predict the extent of multiple dose selegiline exposure.

KEY WORDS: selegiline; site-specific; pharmacokinetics.

INTRODUCTION

Selegiline hydrochloride, [-]-R-N,α-dimethyl-N2-propynl-phenylethylamine hydrochloride (molecular weight 223.75 daltons), is a selective inhibitor of mitochondrial monoamine oxidase type B (MAO-B) at a dosage of 10 mg/day and has been used clinically in combination with L-DOPA in the treatment of Parkinsonism. It is freely soluble in water to an extent of 33 mg/mL. Its partition coefficient (octanol/pH 7.4 buffer) is extremely high indicating a high degree of lipid solubility. Selegiline is rapidly metabolized via N-demethylation and oxidative dealkylation to N-desmethylselegiline (DMS) and L-methamphetamine (MET), respectively. Subsequently, N-desmethylselegiline and L-methamphetamine are further metabolized to L-amphetamine (AMP). Selegiline undergoes significant first pass metabolism following oral administration. Based on a single dose comparison of 10 mg oral versus 1 mg intravenous dosing, the bioavailability of selegiline is less than 10% in healthy male volunteers (1).

Yoshida et al. (2) have shown that both the kidneys and lungs can metabolize selegiline in the rat and Yamlahi et al. (3) have suggested that selegiline is metabolized by the gastrointestinal tract, liver, and lung in the dog. Barrett et al. (4) have recently described the multiple dose pharmacokinetics of orally-administered selegiline hydrochloride and provided supportive evidence for the gastrointestinal contribution to the first-pass metabolism of selegiline. This data confirmed the susceptibility of site-specifically absorbed and metabolized, highly-extracted drugs such as selegiline to food and input rate effects (4). Recent data suggests that both the rate and extent of drug absorption and metabolic fate of selegiline may be influenced by the anatomical/physiological differences between different segments of the intestine (5). These phenomenon may also explain the variability in selegiline plasma levels following oral administration (6–7). Site-dependent absorption and metabolism have been examined using various intestinal absorption techniques for a variety of marketed and investigational drugs (8–11). The present study was conducted to assess the comparative bioavailability of selegiline when administered orally and at three different gastrointestinal sites.

Selegiline is metabolized by cytochrome P450 isozymes 2D6 (12) and 3A4 (L. Z. Benet, personal communication). Mucosal cells of the small intestine are abundant in these P450 isozymes and thus it can be postulated that a portion of the first-pass metabolism of selegiline occurs in the gastrointestinal tract (13). The activity of both P450 3A4 and 2D6 is generally higher in the duodenum and jejunum and it decreases in the lower part of the gastrointestinal tract (14). Thus, depending on the site of absorption of selegiline in the gastrointestinal tract, the disposition of selegiline and its metabolites may be different. An additional objective of this study was to examine the relative disposition of selegiline and its three metabolites DMS, MET, and AMP when selegiline is administered at different sites in the gastrointestinal tract.

METHODS

Study Design and Conduct

This was an open-label, four-way crossover, single dose pharmacokinetic study to compare the bioavailability of selegiline 10 mg administered to healthy young males as a solution by the oral route (in the stomach) and administered by a tungsten-weighted nasoenteric tube with balloon and infusion channels (Zinetics Medical, Inc., Salt Lake City, Utah) to the following three sites: duodenum, jejunum and terminal ileum. The study consisted of four periods with at least 14 days separating each

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period. Subjects were not randomized. Each subject was scheduled to receive the most distal intestinal intubation (terminal ileum) in the first period. In the event that this dosing region was not achieved, the study drug was administered to the attained site (as determined by fluoroscopy) on that study day and the next period was adjusted to reflect the terminal ileal administration. Hence, each subject had three chances for the nasoenteral tube to reach the terminal ileum. Jejunal then duodenal intubations were targeted for periods two and three, respectively, while the oral dose was targeted for period four.

On treatment days in which selegiline was administered nasoenterically, the dosing sites were at the central portion of the duodenum, the central portion of the jejunum, and the distal portion of the small intestine just proximal to the ileal-cecal junction. The nasoenteric tube was inserted approximately 18:00 hours of the evening before dosing. Passage of the nasoenteric tube beyond the duodenum was confirmed by fluoroscopy approximately 2 hours post-insertion prior to inflation of the baloon. A minimum of six fluoroscopy exposures were performed (at least 2 per period). Exposure to levels of fluoroscopic radiation did not exceed 1.5 mSv per subject. While confined to the clinic, no food or beverages (other than water) were consumed by the subjects except during the designated meal periods. Subjects were required to fast at least 12 hours prior to drug administration (0 hour) and four hours following dosing. Standard meals were provided at 4 and approximately 9 hours after dosing and at appropriate times thereafter. Subject demographics and the dosing site allocation schedule are contained in Table I. In each period, selegiline was administered via nasoenteric tube. Infusions were administered over a one minute interval.

The protocol was reviewed by the Phoenix International Life Sciences Institutional Review Board in accordance with the principles and requirements described in "Guidelines on Research Involving Human Subjects" (Medical Research Council of Canada, 1987) and in the U.S. Code of Federal Regulations (21 CFR Part 56). Subjects were judged to be in good health and able and willing to give informed consent. Prior to enrollment and at the completion of the study each subject underwent a complete physical examination, clinical laboratory testing (blood chemistry, hematology, and urinalysis), and 12-lead electrocardiogram. In addition, subjects were screened for inclusion with a urinary screen for drugs of abuse and serum tests for alcohol, HIV antibody, and HB_sAg.

Each subject was queried for the occurrence of adverse experiences prior to the study drug administration, at 4 hours after dosing, and once each morning thereafter during each treatment period. Seated blood pressure and heart rate were recorded at 0 (pre-dose, within 30 minutes of drug administration), 1, 6, 24, 36, 48, 72, 96 and 120 hours post dosing following each treatment phase.

Bioanalytical

Selegiline and metabolites (DMS, AMP, MET) were recovered together from a plasma matrix in a single extraction step. Quantitation of all four analytes was completed via a highly sensitive HPLC/MS/MS method (15). The lower limit of quantitation (LLOQ) using the HPLC/MS/MS human plasma assay for each analyte was the following: 0.01 ng/mL for selegiline, 0.05 ng/mL for DMS, 0.20 ng/mL for AMP and 0.20 ng/mL for MET.

Pharmacokinetics

Plasma concentrations below the LLOQ for each analyte were not used in any pharmacokinetic calculation. Plasma concentrations of selegiline refer to the free base. Actual sampling times were used in all pharmacokinetic calculations. Plasma analyte (selegiline, DMS, AMP, and MET) area under the curve (AUC) up to the last measured time point (AUC_t) after each administration were calculated using a combined log-linear trapezoidal method (linear up to the first point of the terminal phase and log-linear for the terminal phase). The terminal disposition rate constant (λ) was estimated by regression of the terminal log-linear concentration-time points. Terminal disposition half-life was calculated as the quotient of the natural log of 2 and λ. AUC_∞ was calculated from the sum of the truncated area, AUC_t, and the extrapolated area, $AUC_{t-\infty}$, obtained by dividing the last measured analyte plasma concentration by λ. The ratio of metabolite to selegiline AUC_t was calculated for each administration. The bioavailability of selegiline at each

Table I. Subject Demographics and Dosing Allocation of Healthy Males[d] Receiving Single 10 mg Oral Doses of Selegiline Hydrochloride to the Stomach, Duodenum, Jejunum, and Ileum

	Demographics			Site of Dosing			
Subject	Age (yrs)	Weight (kg)	Height (cm)	Period 1	Period 2	Period 3	Period 4
1	23	70.7	180	stomach[a,b]	ileum[a]	duodenum[a]	jejunum
2	25	65.0	186	ileum[a]	duodenum	jejunum	stomach
3	25	79.4	172	ileum[a]	jejunum	stomach[b]	duodenum
4	21	65.6	174	ileum	jejunum	duodenum	stomach
5	27	82.4	176	jejunum[a]	ileum	duodenum	stomach
6	22	65.5	160	jejunum	duodenum	stomach[b]	—[c]
7	19	75.7	178	ileum	jejunum	duodenum	stomach
8	20	63.5	169	jejunum	duodenum	stomach[b]	ileum

[a] Tube inserted via mouth. In all other cases the tube was inserted nasally.
[b] Dosed in stomach via tube instead of administering solution orally.
[c] Ileal site not reached in period 4 for Subject 6.
[d] Mean age = 22.8 ± 2.8 years; mean height = 174.4 ± 7.8 cm; mean weight = 71.0 ± 7.3 kg; all subjects were caucasian.

GI site relative to oral (stomach) administration ($F_{D/PO}$, $F_{J/PO}$, and $F_{I/PO}$) was calculated using the following equation.

$$F_{GI\ Site} = \frac{Dose_{PO} * AUC_{GI\ Site}}{Dose_{GI\ Site} * AUC_{PO}} \quad (1)$$

All pharmacokinetic parameters were calculated using PC SAS for Windows version 6.10 (16).

Statistics

A Student's t-test (two tailed with equal variance assumption) was performed between vital signs at each sampling time compared to baseline determinations. The frequency, type, and severity of adverse experiences and reported laboratory abnormalities were compared across treatments. Statistical analysis was performed on each pharmacokinetic parameter (C_{max}, T_{max}, AUC_t, half-life, and metabolite AUC_t ratios) separately based on a randomized block ANOVA. Statistically significant effects were further examined by pairwise comparisons using Tukey's method of multiple comparisons. Relative bioavailabilities ($F_{D/PO}$, $F_{J/PO}$, and $F_{I/PO}$) of each dosing site relative to oral (stomach) administration were assessed by the two one-sided test procedure (17). The least squares means from the ANOVA were used to construct 90% confidence intervals about each dosing site. All statistical analyses were completed using SAS version 6.10. All tests of significance were performed at the $p = 0.05$ level.

RESULTS

Safety

There were no serious adverse events (AEs) associated with the administration of selegiline hydrochloride. The most common AEs reported were headache, sore throat, and runny nose with 4 reported events each, 4 following oral (stomach) administration and 4 following ileal, 3 following duodenal and 1 following jejunal administration. There did not appear to be any association of clinical AEs with any treatment administered. There were no clinically significant ECG or laboratory abnormalities. Of the eight subjects receiving the solution in the stomach, 4 subjects were dosed via the nasoenteric tube (as opposed to the mouth).

Pharmacokinetics

Selegiline was absorbed equally well following administration to the duodenal, jejunal, or ileal regions of the gastrointestinal tract and to a greater extent (approximately 150% greater) when administered to the stomach. Plasma levels of the subjects receiving the stomach administration via tube versus orally were not different. Maximal selegiline plasma concentrations were achieved in less than 30 minutes at all dosing sites. Mean plasma concentration-time profiles for each analyte at each dosing site are shown in Figure 1. The relative selegiline bioavailability of the intestinal regions with respect to the stomach was 52% (30%, 91%) for the duodenum, 67% (34%, 131%) for the jejunum, and 37% (18%, 73%) for the ileum. There was no difference in selegiline C_{max}, T_{max} or half-life across dosing sites. Table II contains the pharmacokinetic summary of all analytes across dosing site. Relative bioavailabilities and

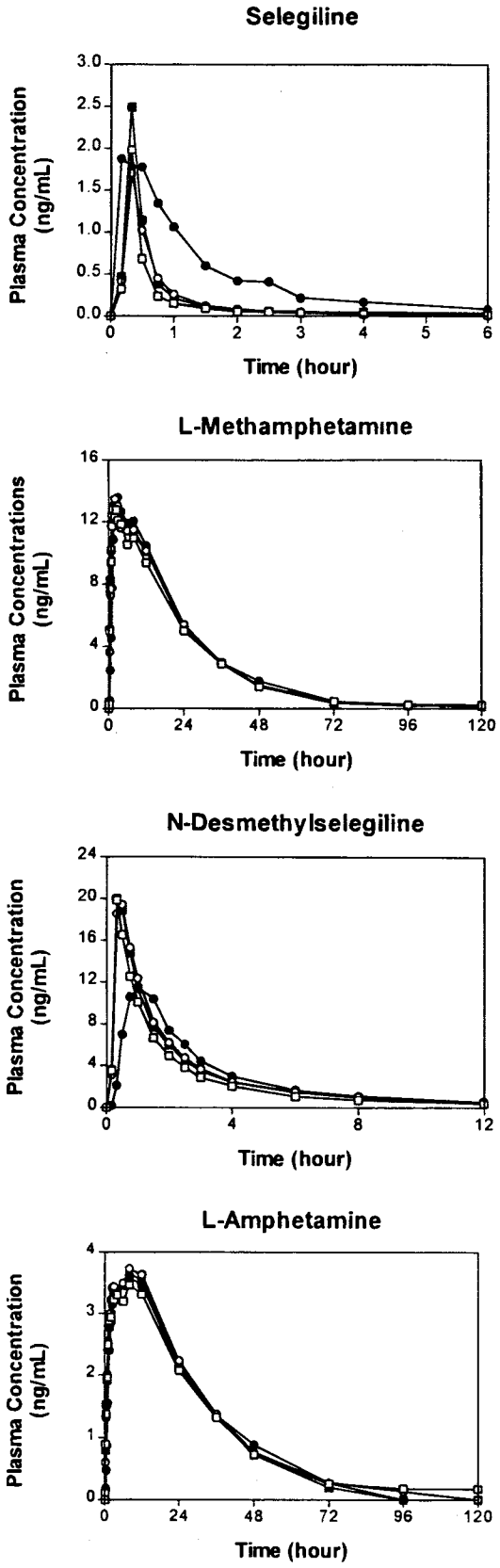

Fig. 1. Mean plasma concentrations of selegiline, N-desmethylselegiline, L-amphetamine, and L-methamphetamine in healthy young males (n = 8) after single 10 mg oral doses of selegiline hydrochloride to the stomach, (●); duodenum, (○); jejunum, (■); and ileum, (□).

Table II. Pharmacokinetic Parameters[a] of Selegiline, DMS, MET, and AMP in Healthy Males (n = 8[b]) Receiving Single 10 mg Oral Doses of Selegiline Hydrochloride to the Stomach, Duodenum, Jejunum and Ileum

Analyte	Parameter	Dosing Site				Pairwise Comparisons[d]
		Stomach (S)	Duodenum (D)	Jejunum (J)	Ileum[b] (I)	
Selegiline	C_{max} (ng/mL)	2.1 (1.4)	1.7 (0.7)	2.5 (1.8)	2.0 (1.4)	[S D J I]
	T_{max} (hr)	0.4 (0.2)	0.3 —	0.3 —	0.3 —	[S D J I]
	AUC_t (ng*hr/mL)	3.1 (2.5)	1.1 (0.5)	1.2 (0.8)	0.8 (0.5)	[S] [D J I]
	$t_{1/2}$ (hr)	4.5 (2.3)	3.4 (2.8)	3.5 (1.1)	2.3 (1.2)	[S D J I]
DMS	C_{max} (ng/mL)	15.3 (5.6)	20.3 (5.1)	20.9 (4.3)	20.9 (7.6)	[S D J I]
	T_{max} (hr)	1.2 (0.6)	0.4 (0.1)	0.4 (0.1)	0.4 (0.1)	[S] [D J I]
	AUC_t (ng*hr/mL)	43.6 (15.5)	43.8 (12.6)	40.4 (8.7)	34.4 (8.1)	[I] [S D J]
	$t_{1/2}$ (hr)	6.0 (2.5)	6.4 (3.8)	5.3 (1.7)	5.2 (1.8)	[S D J I]
	$R_{DMS:SEL}$[c]	39.1 (51.4)	53.0 (26.3)	48.6 (25.5)	60.6 (42.0)	[S D J I]
MET	C_{max} (ng/mL)	14.9 (2.9)	14.2 (1.9)	13.4 (1.6)	13.8 (2.3)	[S D J I]
	T_{max} (hr)	2.6 (0.7)	2.8 (2.2)	2.7 (1.7)	1.6 (0.9)	[S D J I]
	AUC_t (ng*hr/mL)	330.3 (97.1)	326.5 (108.1)	318.5 (76.3)	314.4 (117.9)	[S D J I]
	$t_{1/2}$ (hr)	14.2 (2.0)	14.3 (3.6)	12.9 (2.5)	15.8 (3.8)	[S D J] [S D I]
	$R_{MET:SEL}$[c]	596.3 (1155.5)	614.8 (781.5)	509.2 (399.4)	829.7 (1025.2)	[S D J I]
AMP	C_{max} (ng/mL)	3.8 (0.5)	3.8 (0.6)	3.7 (0.4)	3.5 (0.6)	[S D J I]
	T_{max} (hr)	8.3 (3.8)	6.3 (3.4)	8.1 (2.9)	7.1 (3.3)	[S D J I]
	AUC_t (ng*hr/mL)	118.5 (21.4)	120.9 (28.9)	114.6 (22.0)	113.5 (32.3)	[S D J I]
	$t_{1/2}$ (hr)	15.9 (1.8)	16.2 (2.2)	14.9 (3.1)	16.9 (5.1)	[S D J I]
	$R_{AMP:SEL}$[c]	212.8 (369.0)	238.9 (278.3)	197.5 (138.4)	310.9 (343.4)	[S D J I]

[a] Mean with standard deviation in parentheses.
[b] Summary statistics for lieal administration are based on n = 7 subjects completing this treatment.
[c] Ratio of metabolite AUC_t to selegiline AUC_t adjusted for molecular weight.
[d] Treatment groups in brackets are equivalent ($p > 0.05$).

90% confidence intervals of intestinal sites relative to the stomach administration for each analyte are shown in Table III.

While mean peak DMS concentration was lower following selegiline administration to the stomach, this was not significantly different from peak concentrations obtained following intestinal administrations (15.3 versus approximately 20.5 ng/mL). This may be due to the dependence of C_{max} on sampling time and the limitation of the small sample size used in this study. The time to peak DMS concentration was shifted following oral selegiline administration (1.2 versus 0.4 hours). DMS exposure (AUC_t) following ileal selegiline administration was lower (approximately 33%) than that obtained following proximal dosing sites. The DMS: selegiline AUC ratio, however, was equivalent across dosing sites. Both MET and AMP yielded equivalent plasma levels following all administrations. There was no difference in MET or AMP exposure relative to selegiline following any of the stomach or intestinal administrations.

Table III. Relative Bioavailabilities ($F_{D/PO}$, $F_{J/PO}$, and $F_{I/PO}$) and 90% Confidence Intervals About Selegiline Log-transformed AUC_t

	Least Squares Mean Estimate	90% Confidence Interval	
		Lower	Upper
Duodenum	0.52	0.30	0.91
Jejunum	0.67	0.34	1.31
Ileum	0.37	0.18	0.73

A significant pairwise comparison in MET half-life was considered spurious (Table II).

Figure 2 shows the mean metabolite to selegiline plasma concentration profiles for stomach, duodenal, jejunal, and ileal administration sites. These profiles document the equivalence of intestinal dosing sites with respect to MET and AMP disposition. AMP and MET to selegiline plasma ratios are lowest following administration to the stomach. MET: selegiline profiles reach 500 to 750 fold following intestinal intubation while ratios following stomach administration are less than 400 fold. Similarly, AMP: selegiline levels are less following stomach administration compared to intestinal dosing sites. Stomach DMS: selegiline plasma ratios were similar to those obtained following intestinal administration.

DISCUSSION

Selegiline therapy in Parkinson's Disease is expected to continue throughout treatment with L-DOPA. The recommended regimen is twice daily at breakfast and lunch. As sustained release Sinemet (carbidopa/levodopa) formulations have been shown to be both desirable and advantageous, the development of similar sustained release adjuncts such as selegiline hydrochloride would likewise be desirable. A selegiline hydrochloride pulsatile release capsule containing an immediate release 5 mg dose and a second 5 mg dose delivered approximately 4 hours post initial dose has been recently examined in man (5). A comparison of the plasma profiles from this study with historical data from the conventional immediate

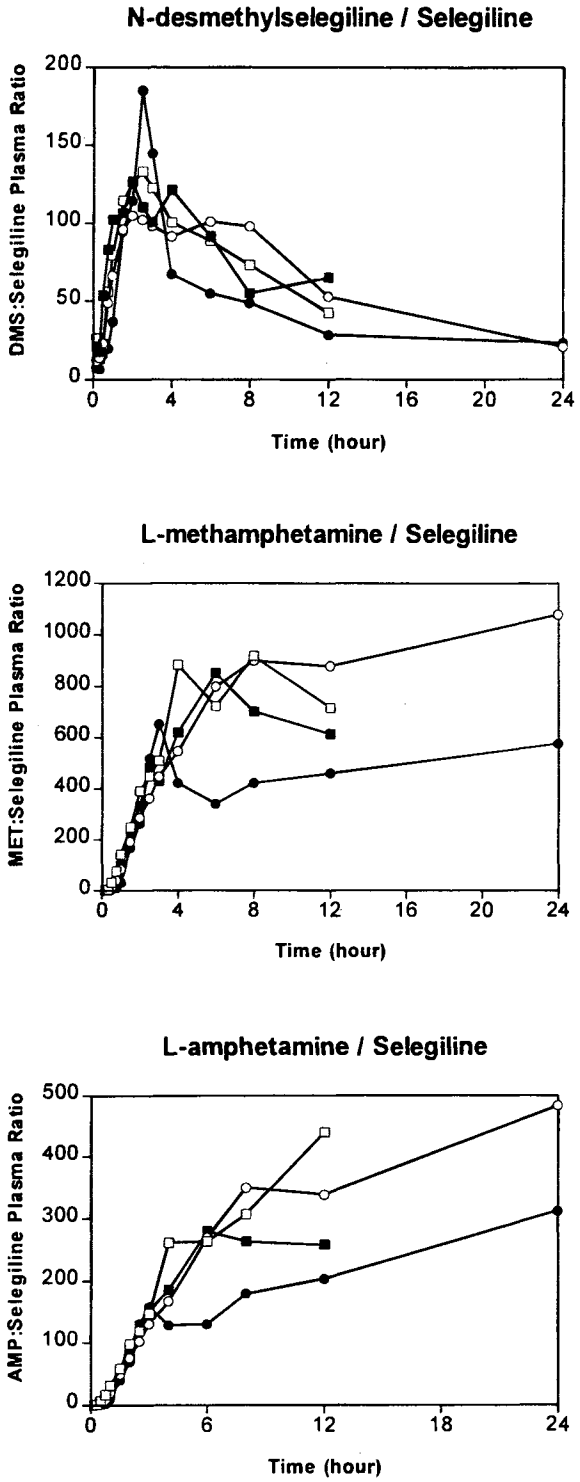

Fig. 2. Mean metabolite:selegiline plasma ratio profiles for N-DMS/SEL, L-AMP/SEL, L-MET/SEL in healthy young males after single 10 mg oral doses of selegiline hydrochloride to the stomach (●), duodenum (○) jejunum (■) and ileum (□).

release tablet given twice daily suggested that presystemic metabolism may preclude the development of sustained release selegiline hydrochloride formulations required to be bioequivalent to the twice daily regimen of the conventional tablet (5). The current study was designed to assess the feasibility of sustained release selegiline hydrochloride formulations and determine if such formulations would be susceptible to site-specific differences in selegiline absorption and presystemic metabolism.

Selegiline is completely absorbed when given as an oral solution (18,19). In this study, the oral solution was used as the reference in estimating the relative bioavailability at duodenal, jejunal, and ileal sites along the gastrointestinal tract. Results indicate that selegiline was absorbed equally well following gastrointestinal administration to the duodenum, jejunum, and ileum. Hence, a sustained release formulation should be viable from an absorption standpoint. While selegiline exposure as measured by AUC_t was greater after stomach than intestinal administration, metabolite exposure (DMS, MET, and AMP) was comparable between stomach and intestinal administrations except for DMS at the ileal site which was approximately 33% less than the exposure obtained at other dosing sites. Reduced DMS exposure following selegiline administration to the ileum can presumably be explained by the reduction in cytochrome P450 isozyme density at distal gastrointestinal sites. DMS AUC_t following ileal intubation, however, could be explained by selegiline levels which were generally (albeit not statistically significant) lower. It is uncertain if, similar to results with a pulsatile selegiline hydrochloride delivery system (5), DMS systemic exposure would be reduced following sustained release administration. Half-life estimates for selegiline and metabolites were unaffected by site of administration. Compared with results with the antidepressant and P450 substrate nefazodone (10) from a similar experimental approach, selegiline metabolism may be less influenced by site-specific processes. Unlike nefazodone, selegiline's metabolites AMP and MET are not significantly lower following distal administration. While there is a presystemic component to DMS formation, the fraction of DMS systemic exposure due to presystemic metabolism is likely less than the proportion of first-pass metabolism of nefazodone which occurs in the gastrointestinal tract.

The divergence between intestinal and stomach dosing sites with respect to AMP and MET metabolic profiles occured at approximately 3 hours post dose (after selegiline absorption has been completed) suggesting that less selegiline was available for conversion to MET and subsequently to AMP. Differences in metabolic profiles at later time points likely reflects the low and variable selegiline levels at these times and should not suggest metabolic differences across intestinal sites with time. Despite selegiline exposure being greater following administration to the stomach, systemic AMP and MET exposure was unaffected consistent with the hepatic formation of both metabolites. Given the variability in selegiline plasma levels following single dose oral administration, it was difficult to separate first-pass versus gastrointestinal presystemic metabolic differences without more invasive study designs. The first-pass extraction of selegiline is substantial (1,19). As DMS formation along the gastrointestinal site is thought to occur in proximal regions, DMS exposure following distal administration may require hepatic compensation. These observations would require confirmation from multiple dose studies as presystemic metabolism is likely to be saturable (4).

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