Dose linearity study of selegiline pharmacokinetics after oral administration: evidence for strong drug interaction with female sex steroids

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Aims The purpose of this study was to characterize the dose relationship of selegiline and desmethylselegiline pharmacokinetics within the selegiline dose range from 5 to 40 mg.

Methods Eight female subjects, of whom four were using oral contraceptives, ingested a single dose of 5 mg, 10 mg, 20 mg or 40 mg of selegiline HCl in an open four-period randomized study. Concentrations of selegiline and desmethylselegiline in serum were measured by gas chromatography for 5 h. As it became evident that the use of oral steroids had a drastic effect on selegiline concentrations, the pharmacokinetic analyses were performed separately for oral contraceptive users and those not receiving any concomitant medication.

Results The total AUC and $C_{\max}$ of selegiline were 10- to 20-fold higher in those subjects taking oral steroids compared with subjects with no concomitant medication; this finding was consistent and statistically significant at all the four dose levels. The dose linearity of selegiline pharmacokinetics failed to be demonstrated in both groups. The AUC and $C_{\max}$ of desmethylselegiline were only moderately higher (about 1.5-fold; $P = \text{NS}$ at each dose level) in the subjects taking oral steroids than in those not receiving concomitant medication. The AUC values of desmethylselegiline increased in a dose linear manner in subjects with no concomitant medication, but not in the oral steroid group. The metabolic ratio (AUC(desmethylselegiline)/AUC(selegiline)) was several-fold lower in the group receiving oral steroids compared with the no-concomitant-medication group ($P < 0.005$ at all the four dose levels).

Conclusions Concomitant use of oral contraceptives caused a drastic (20-fold) increase in the oral bioavailability of selegiline. The highly significant difference in the metabolic ratio between the groups provides evidence that the mechanism of the interaction between selegiline and female sex steroids involves reduced *N*-demethylation of selegiline. The present results suggest that concomitant use of selegiline with exogenous female sex steroids should be avoided or the dosage of selegiline should be reduced in order to minimize the risks of selegiline related adverse drug reactions.

Keywords: desmethylselegiline, female sex steroids, interaction, pharmacokinetics, selegiline

Introduction

Selegiline (1-deprenyl) is used in the treatment of Parkinson's disease as a monotherapy or in combination with levodopa [1–3]. The pharmacological action of

selegiline is based on the selective and irreversible inhibition of monoamine oxidase (MAO) type B, resulting in the decreased degradation of dopamine in the central nervous system [4]. Also, the main metabolite of selegiline, desmethylselegiline, has been shown to have some inhibitory effect on MAO-B [5, 6]. The therapeutic daily dose of selegiline HCl in the treatment of Parkinson's disease is 5 or 10 mg; however, much higher doses (30–40 mg daily) of selegiline have been used for the treatment of depression and narcolepsy [7–9].

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Received 13 July 1998, accepted 11 October 1998.

The pharmacokinetics of selegiline after oral administration are characterized by a rapid absorption and an extensive first-pass metabolism, which limits the bioavailability of the parent compound to about 10% [10]. Concentrations of desmethylselegiline in serum are markedly higher compared with those of selegiline after administration of a single oral dose of selegiline. Because of the low serum selegiline concentrations and the large interindividual variation in selegiline pharmacokinetics, the results of earlier pharmacokinetic studies on selegiline have been often based on the serum levels of desmethylselegiline.

Recently, more sensitive assay methods for the determination of selegiline and desmethylselegiline in serum have been developed [11]. This offers more reliable characterization of the pharmacokinetics of both the pharmacologically active compounds, selegiline and desmethylselegiline. Furthermore, with increasing serum selegiline concentrations the selectivity to MAO-B can be lost [12]. The inhibition of both MAO-A and MAO-B could lead to undesired drug effects, such as tyramine potentiating effects or the serotonin syndrome. As very little is known about the pharmacokinetics of selegiline after higher (>10 mg) oral doses it was considered important to characterize the dose relationship of selegiline and desmethylselegiline pharmacokinetics within the selegiline dose range from 5 to 40 mg.

Methods

Subjects and ethics

Eight female subjects (aged between 20 and 27 years and weighing between 56 and 75 kg) participated in the study. The good health of the subjects was ensured by medical history, physical examination and results from standard clinical chemistry and haematological tests, prior to the study. All the subjects were nonsmokers and with the exception of four subjects, who took oral contraceptives (three subjects used the combination of gestodene 75 µg/ethinylestradiol 30 µg and one subject a triphasic preparation containing levonorgestrel 50–125 µg/ethinylestradiol 30–40 µg), none of the subjects was taking any prescribed or nonprescribed drugs for 2 weeks before or during the study. The demographic characteristics of the subjects using oral contraceptives did not differ from those not receiving any concomitant medications. The subjects received both verbal and written information on the study, and written informed consent was obtained. The study protocol was approved by the joint Ethics Committee of the Faculty of Medicine, University of Turku and Turku University Central Hospital.

Protocol

The subjects received a single dose of 5 mg (one Eldepryl[®], 5 mg tablet, Orion Corporation, Orion Pharma), 10 mg (two tablets), 20 mg (four tablets) or 40 mg (eight tablets) of selegiline hydrochloride in an open 4-period randomized study with a washout period of at least 2 weeks between the phases. The selegiline dose was ingested on an empty stomach (after an overnight fast) with 200 ml of water. Fasting was continued for 3 h after drug ingestion. Use of food containing large amounts of tyramine was restricted within 1 week before and 2 weeks after the study drug administration. Alcohol intake was forbidden for 2 days before and 24 h after selegiline administration. The subjects were asked to refrain from strenuous physical exercise during the fasting period and for 24 h after the drug administration.

Blood samples (10 ml) were drawn through an antecubital venous cannula at 0, 15, 30 and 45 min and 1, 1.5, 2, 2.5, 3, 4 and 5 h after drug ingestion. The blood samples were centrifuged and serum was separated for the analysis of selegiline and desmethylselegiline concentrations. The samples were stored at -20°C until analysed. Blood pressure and heart rate were recorded in sitting position after each blood sampling until 5 h postdose.

Analytical methods

Concentrations of selegiline and desmethylselegiline in serum were determined by a validated gas chromatographic method employing a nitrogen selective detection [13]. The limit of quantification was defined as 0.1 ng ml^{-1} for selegiline and 0.2 ng ml^{-1} for desmethylselegiline with an intra-assay precision of 13.2% and 14.8%, respectively. Assessment of interassay precision, based on quality control samples, yielded relative standard deviation less than 10% at selegiline concentrations of 0.5, 2, 8 and 20 ng ml^{-1} and desmethylselegiline concentrations of 0.8, 8, 32 and 80 ng ml^{-1} .

Data analysis

The pharmacokinetic parameters for selegiline and desmethylselegiline were calculated by standard noncompartmental methods. The rate and extent of selegiline absorption after each dosage regimen were characterized by determining the peak selegiline and desmethylselegiline concentration in serum (C_{max}), time to peak (t_{max}) and the area under the serum concentration-time curve from 0 to infinity (AUC), using the linear trapezoidal rule to the last sampling time (5 h) and extrapolation to infinity by dividing the concentration at 5 h (if not below

quantification limit) by elimination rate constant. The half-lives ($t_{1/2}$) of selegiline and desmethylselegiline were estimated by the least squares regression analysis of the terminal linear part of log concentration–time curve. The metabolic ratio (MR) was assessed by dividing the AUC of desmethylselegiline by the AUC of selegiline.

Differences in the relative bioavailability (AUC) among the four different selegiline HCl doses was investigated separately in subjects with no concomitant medication ($n=4$) and in subjects taking oral contraceptive steroids ($n=4$) by two-way analysis of variance (ANOVA; suitable for cross-over design) after appropriate dose corrections; two-way anova was also used for the $t_{1/2}$ data. Student's t -test (two-tailed) for unpaired data was used to find differences in the parameters AUC, $C_{\max}$ and $t_{1/2}$ between the steroid group and the no-concomitant-medication group at each dose level. The corresponding nonparametric tests were used for the $t_{\max}$ data. Similarly, nonparametric testing (Mann–Whitney) was used for MR data due to unequal group variances. The AUC, $C_{\max}$ and $t_{1/2}$ data were log-transformed prior to statistical testing. Ninety-five percentage confidence intervals were calculated for the ratio of the two means for the main endpoints using log transformed data (Table 1). P values <0.05 were considered statistically significant.

Results

All the subjects completed the study according to the protocol. No adverse effects were reported by any of the subjects at any dose level. Also, no clinically relevant changes in blood pressure or heart rate were observed within 5 h after selegiline intake in any of the subjects.

Selegiline pharmacokinetics

The selegiline AUC was significantly higher at all four dose levels in those subjects using oral contraceptive steroids compared with the subjects not using any concomitant medication (Table 1, Figure 1). For example, at the 10-mg dose level, which is most commonly used in the treatment of Parkinson's disease, there was about a 20-fold difference in the mean selegiline AUC (Figure 1). Similarly, the median $C_{\max}$ was more than 10-fold higher in the group taking oral steroids (Table 1; $P=0.002$). However, the $t_{\max}$ was not affected by concomitant oral steroids at any of the four dose levels (Table 1; $P=NS$). Due to low concentrations, the elimination half-life of selegiline could not be reliably calculated for all the subjects.

The AUC of selegiline increased in a nonlinear manner on increasing the dose as evidenced by the significant difference in dose corrected AUC values among the different dosage regimens both in the group using oral

steroids ($P=0.001$) and in the group not receiving any concomitant medication ($P<0.001$).

Desmethylselegiline pharmacokinetics

The AUC values of desmethylselegiline were somewhat higher in the group using concomitant oral contraceptives compared with those not receiving any concomitant medication (Table 1). This finding was consistent at all the four dose levels, but was much smaller in magnitude compared with what was found with selegiline, and did not reach statistical significance. Also, no significant difference was found in the $C_{\max}$ or $t_{\max}$ between the groups (Table 1). The elimination half-life of desmethylselegiline was about 1.5 h in the group that received no concomitant medication and was not dependent on the selegiline dose ($P=0.90$; Table 1). In the group using oral steroids the $t_{1/2}$ was somewhat longer at each dose level, but reached statistical significance only after the 40mg dose ($P=0.02$).

The AUC of desmethylselegiline increased in a dose-linear manner in the group that received no concomitant medications, as evidenced by the nonsignificant difference in dose corrected AUC values among the different dosage regimens ($P=0.11$). In the oral steroid group the increase in the AUC values was nonlinear ($P=0.02$).

Metabolic ratio

The metabolic ratio, calculated as $AUC(\text{desmethylselegiline})/AUC(\text{selegiline})$, was decreased in both groups on increasing the dose, suggesting saturation of the first-pass metabolism of selegiline to desmethylselegiline on increasing the dose of selegiline (Figure 2). The metabolic ratio was several-fold lower in the group receiving oral contraceptive steroids compared with the group that did not take any concomitant medications (Figure 2; $P<0.005$ at all dose levels).

Discussion

The mean AUC of selegiline was about 20-fold greater in those four women that used concomitant oral contraceptive steroids compared with subjects with no concomitant drugs. Importantly, this finding was evident in all the four subjects using oral contraceptives, although there was large interindividual variation among the subjects in both groups. Also, a similar difference was found in the selegiline $C_{\max}$ between the groups.

N-demethylation of selegiline is considered to be a major metabolic pathway of selegiline elimination and it has been postulated that cytochrome P450-enzymes may be responsible for catalysing this reaction [14, 15]. The pronounced difference in the metabolic ratio between

Table 1 Pharmacokinetic parameters of selegiline and desmethylselegiline after oral administration of 5, 10, 20 or 40 mg selegiline HCl to healthy volunteers with no concomitant medication ($n=4$) and with concomitant oral steroids ($n=4$).

Parameter	No concomitant medication				Concomitant oral steroids			
	5 mg	10 mg	20 mg	40 mg	5 mg	10 mg	20 mg	40 mg
<i>Selegiline</i>								
AUC (ng ml ⁻¹ h)	0.04 (0.04–0.08)	0.5 (0.3–0.7)	0.9 (0.5–2.1)	5.3 (3.4–6.5)	1.8 (0.6–5.3)†	11 (1.5–13)★	22 (16–51)†	89 (51–179)†
95% CI for ratio of means					(11, 131)	(3.6, 54)	(9.7, 70)	(7.5, 42)
C _{max} (ng ml ⁻¹)	0.05 (0.05–0.2)	0.8 (0.5–1.1)	1.0 (0.6–2.0)	5.4 (1.6–11)	2.8 (1.3–7.5)†	8.9 (2.7–22)★	15 (8.1–44)†	101 (12–203)★
95% CI for ratio of means					(10, 148)	(3.6, 34)	(5.2, 51)	(1.7, 118)
t _{max} (h)	0.75 (0.75–0.75)	0.75 (0.5–1.5)	0.75 (0.5–0.75)	1.3 (0.5–3.0)	0.5 (0.5–0.75)	0.5 (0.5–0.75)	0.5 (0.5–2.5)	0.5 (0.5–0.75)
t _{1/2} (h)	n.a.	n.a.	n.a.	n.a.	n.a.	1.8 (1.5–4.3) ^a	1.4 (1.0–1.9) ^a	1.6 (1.5–2.1)
<i>Desmethylselegiline</i>								
AUC (ng ml ⁻¹ h)	12 (5.9–18)	31 (23–41)	63 (50–98)	115 (102–151)	18 (12–32)	33 (26–86)	102 (64–138)	269 (178–379)
95% CI for ratio of means					(0.8, 3.6)	(0.6, 2.6)	(0.8, 2.8)	(0.6, 2.9)
C _{max} (ng ml ⁻¹)	8.6 (2.7–11)	21 (15–27)	39 (27–50)	64 (24–73)	9.4 (7.3–16)	14 (8.5–57)	29 (24–80)	64 (53–114)
95% CI for ratio of means					(0.6, 3.6)	(0.3, 2.5)	(0.5, 1.9)	(0.5, 3.6)
t _{max} (h)	0.75 (0.5–1.0)	0.63 (0.5–1.0)	0.75 (0.5–2.0)	0.88 (0.75–3.0)	0.75 (0.5–1.0)	0.5 (0.5–0.75)	0.75 (0.75–3.0)	0.88 (0.5–2.5)
t _{1/2} (h)	1.4 (1.2–2.7)	1.6 (1.3–1.8)	1.7 (1.6–1.9)	1.5 (1.3–1.7)	1.9 (1.3–3.4)	1.9 (1.8–3.0)	2.1 (1.7–3.0)	2.8 (2.5–5.0)★
95% CI for ratio of means					(0.6, 2.5)	(0.9, 1.9)	(0.9, 1.8)	(1.4, 3.1)

All data are given as median (range); n.a., not assessable; ^a $n=3$; CI, confidence interval. Statistically significant difference compared with the no-concomitant-medication group, * $P<0.05$; † $P<0.001$.

Figure 1 Selegiline AUC values in subjects with no concomitant medication (□, $n=4$) and in subjects using concomitant oral steroids (▤, $n=4$), after administration of four different single oral doses of selegiline hydrochloride (mean values).

Figure 2 Metabolic ratio (desmethylselegiline AUC/selegiline AUC) in subjects with no concomitant medication (●, $n=4$) and in subjects using concomitant oral steroids (○, $n=4$), after administration of four different single oral doses of selegiline hydrochloride (mean values $\pm$ s.d.).

the groups at each dose level suggests that the mechanism of the interaction between oral female sex steroids and selegiline is inhibition of *N*-demethylation of selegiline to desmethylselegiline.

Heinonen *et al.* [10] have reported that desmethylselegiline concentrations are markedly higher after oral administration of selegiline compared with i.v. administration. This, together with the low oral bioavailability of selegiline, gives evidence that most of the formation of desmethylselegiline takes place during the first-pass phase in the gut wall and/or in the liver after oral administration. Accordingly, strong inhibition of the first-pass metabolism can be expected to induce marked changes in selegiline bioavailability as was shown in the present study.

Allowing the use of oral contraceptives in half of the women participating in the study evidently led to the failure in assessing the dose-linearity of selegiline pharmacokinetics. This was quite unpredictable since there is limited *in vivo* evidence for the influence of oral contraceptive steroids on drug metabolism [16–19]. Gestodene and other progestogens are known strong inhibitors of CYP3 A and P-glycoprotein *in vitro* [20–22], but the low daily doses of steroid hormones administered in oral contraceptives do not have a major impact on drug metabolism *in vivo*. The present results, however, provide evidence that in some instances the inhibitory effect can be quite drastic. Therefore, women using exogenous female sex steroids should not be routinely enrolled as subjects to clinical drug trials. It should be noted that the design of the present study could not control the possible changes in selegiline pharmacokinetics due to menstrual cycle.

A dose linear increase was observed in the AUC of desmethylselegiline in those four subjects that did not take oral steroids. In subjects receiving oral steroids, the increase in the AUC of desmethylselegiline was nonlinear, suggesting that further metabolism of desmethylselegiline to (+)-amphetamine [14] could also have been inhibited. This effect was, however, quite small and since the MAO-B inhibitory effect of desmethylselegiline is considerably lower than that of selegiline, it is not likely to produce significant changes in pharmacodynamic effects.

In clinical practice, concomitant use of selegiline and oral contraceptive steroids is very unlikely. However, even higher daily doses of, e.g. levonorgestrel are used in hormone replacement therapy (HRT) in postmenopausal women. In this patient group the prevalence of Parkinson's disease is considerably higher as is the likelihood of concomitant use of selegiline and HRT.

The marked increase in the bioavailability of selegiline as was observed in subjects using oral contraceptive steroids could lead to loss of selective inhibition of MAO-B. This can lead to hypertensive reactions ('cheese effect') after intake of amines, especially tyramine, or serotonin syndrome in the presence of serotonergic drugs, such as SSRIs or tricyclics. In an intravenous tyramine test, selegiline, at a daily dose of 60 mg, has been reported to cause a 22-fold increase in tyramine sensitivity [23].

In the present study, the subjects followed a low-tyramine diet and no clinically relevant changes were observed in the heart rate or blood pressure.

In this study, a marked (20-fold) difference was found in the oral bioavailability of selegiline between subjects using oral contraceptive steroids and subjects not using any concomitant medication in addition to selegiline. The mechanism of this interaction involves reduced *N*-demethylation of selegiline, but further information is needed to characterize the metabolic pathways that are affected. The present results suggest that concomitant use of selegiline with exogenous female sex steroids should be avoided or the dosage of selegiline should be reduced in order to minimize the risks of selegiline related adverse drug reactions.

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