

Pharmacokinetic–Pharmacodynamic Interaction Between Nebicapone and Controlled-Release Levodopa/Benserazide: A Single-Center, Phase I, Double-Blind, Randomized, Placebo-Controlled, Four-Way Crossover Study in Healthy Subjects

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ABSTRACT

Background: Nebicapone is a reversible catechol-O-methyltransferase (COMT) inhibitor. Coadministration of a COMT inhibitor with levodopa and a dopa-decarboxylase inhibitor (carbidopa or benserazide) increases levodopa exposure and its therapeutic effect.

Objectives: The primary objective of this study was to investigate the effect of nebicapone (50, 100, and 200 mg), compared with placebo, on levodopa pharmacokinetics when coadministered with a single dose of controlled-release levodopa 100 mg/benserazide 25 mg. The secondary objectives were to investigate the effect of nebicapone on the erythrocyte-soluble COMT (S-COMT) activity and on the plasma levels of the levodopa 3-O-methylated metabolite (3-O-methyldopa [3-OMD]). Nebicapone's tolerability was also assessed.

Methods: This was a single-center, Phase I, double-blind, randomized, placebo-controlled, 4-way crossover study conducted in healthy adult volunteers. Each of the 4 single-dose treatment periods were separated by a washout period of ≥ 5 days. During the different treatment periods, subjects received a single dose of controlled-release levodopa 100 mg/benserazide 100/25 mg concomitantly with nebicapone 50, 100, and 200 mg or placebo. Plasma concentrations of nebicapone, levodopa, and 3-OMD were determined by HPLC. Blood samples (7 mL) for determination of plasma concentrations of levodopa, 3-OMD,

and nebicapone, as well as for the assay of S-COMT activity, were collected in potassium EDTA test tubes at the following times: predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours postdose. S-COMT activity was assessed as the amount of metanephrine formed by the action of S-COMT on an epinephrine substrate. Spontaneously reported clinical adverse events (AEs) were monitored throughout the study period.

Results: Sixteen subjects (8 females, 8 males; mean [SD] age, 26.13 [6.29] years; weight, 69.4 [12.4] kg; body mass index, 24.0 [3.0] kg/m²) completed the 4 treatment periods and had data available for pharmacokinetic and pharmacodynamic analyses. Compared with placebo, levodopa C_{max} increased 25%, 30%, and 34%, and AUC increased 14%, 37%, and 42% after administration of nebicapone 50, 100, and 200 mg, respectively. After administration of nebicapone 50, 100, and 200 mg, 3-OMD C_{max} decreased 44%, 57%, and 58%, and 3-OMD AUC_{0-∞} decreased 33%, 37%, and 45%, respectively, compared with placebo. Extent of exposure to levodopa, as assessed by using AUC_{0-t}, increased with all doses of nebicapone in relationship to placebo, but the difference did not reach statistical significance. This may be related to a rela-

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tively high intersubject variability: %CVs ranged from 48.0% with nebicapone 100 mg to 66.8% with placebo. Maximum S-COMT inhibition by nebicapone occurred at ~1.5 hours postdose and ranged from 57% with nebicapone 50 mg to 74% with nebicapone 200 mg. There was an inverse correlation between plasma concentrations of nebicapone and S-COMT activity; T_{\max} of nebicapone plasma concentrations and time to occurrence of the maximum inhibition of S-COMT activity appeared to correlate. Nineteen AEs were reported; 8 were assessed by the investigator as possibly related to treatments. All AEs were mild in severity. There were no serious AEs or discontinuations due to AEs. No abnormalities in liver enzyme levels were found.

Conclusions: When administered concomitantly with a single dose of controlled-release levodopa 100 mg/benserazide 100/25 mg, single doses of nebicapone 50, 100, and 200 mg were well tolerated in these healthy adult volunteers and dose dependently inhibited S-COMT activity and reduced 3-OMD formation compared with placebo. However, there was no significant difference in levodopa bioavailability. (*Clin Ther.* 2009;31:XXX-XXX) © 2009 Excerpta Medica Inc.

Key words: catechol-O-methyltransferase, COMT inhibition, pharmacokinetics, pharmacodynamics, levodopa/benserazide, nebicapone, Parkinson's disease.

INTRODUCTION

Levodopa remains the most effective symptomatic treatment for Parkinson's disease (PD), but patients can develop motor complications with long-term treatment and disease progression.¹ After oral administration, levodopa is extensively metabolized in the periphery by dopa-decarboxylase (DDC) and catechol-O-methyltransferase (COMT), so that only 1% of an oral dose reaches the brain and is converted into dopamine.² Coadministration of levodopa with a DDC inhibitor such as benserazide or carbidopa effectively decreases the levodopa decarboxylation, but ~90% is converted by COMT to the 3-O-methylated metabolite (3-O-methyldopa [3-OMD]).² One suggested treatment option is the use of COMT inhibitors, which decrease the levodopa methylation to 3-OMD, increase levodopa systemic bioavailability, and provide more continuous dopaminergic stimulation in levodopa-treated PD patients, thus reducing

motor fluctuations.³ An alternative strategy to prevent motor fluctuations in PD patients is the use of dopamine agonists.⁴

Presently, 2 COMT inhibitors are used in combination with levodopa/benserazide or levodopa/carbidopa in patients with levodopa-responsive idiopathic PD and motor fluctuations: tolcapone and entacapone. These agents differ slightly in their basic pharmacology.⁵ The use of tolcapone requires monitoring of liver function⁶ due to the identification, during the post-marketing surveillance period, of cases of fatal hepatic toxicity in patients taking this drug. Tolcapone is thus recommended for the management of fluctuating PD in those patients who have failed to respond with other therapies.⁷ Although entacapone is better tolerated than tolcapone,^{8,9} it is less efficacious, probably because of its lower oral bioavailability (~35% with entacapone and 70% with tolcapone after oral administration) and shorter and less sustained COMT inhibitory activity.² Therefore, new COMT inhibitors that could present a better therapeutic profile are clearly needed.⁵

Nebicapone is a reversible COMT inhibitor^{10,11} currently being developed for use as an adjunct to levodopa plus a DDC inhibitor in the treatment of PD. In single-dose and multiple-dose studies in healthy male subjects,^{12,13} C_{\max} values for nebicapone were attained 0.5 to 2.5 hours postdose. Thereafter, plasma concentrations declined, with an $t_{1/2}$ of ~2 to 4 hours. Pharmacokinetics appeared to be linear and dose proportional, and steady-state plasma nebicapone concentrations occurred by day 4 of twice- or thrice-daily administration.¹³ In early proof-of-concept studies using nebicapone and immediate-release levodopa/carbidopa¹⁴ or immediate-release levodopa/benserazide,¹⁵ healthy subjects receiving single doses of nebicapone 50, 100, 200, and 400 mg experienced increased systemic exposure to levodopa, but no additional benefits were seen with the 400-mg dose compared with the 200-mg dose. A Phase IIA study¹⁶ in 16 PD patients treated with immediate-release levodopa/carbidopa, 4 to 6 times per day, reported that patients receiving nebicapone 75 and 150 mg had significantly decreased COMT activity and increased exposure to levodopa. Effects of nebicapone 75 mg on the COMT activity, levodopa pharmacokinetics, and patient's motor response were similar to that of entacapone 200 mg; nebicapone 150 mg tended to be superior to entacapone 200 mg.¹⁶

The primary objective of the present study was to investigate the effect of 3 single oral doses of nebicapone (50, 100, and 200 mg), compared with placebo, on levodopa pharmacokinetics when coadministered with a single dose of controlled-release levodopa 100 mg/benserazide 25 mg.* Secondly, this study aimed to investigate the effect of single doses of nebicapone on the erythrocyte-soluble COMT (S-COMT) activity and on the plasma levels of 3-OMD, to characterize its pharmacokinetics, and to assess its tolerability after coadministration with controlled-release levodopa 100 mg/benserazide 25 mg.

*Trademark: Madopar® HBS 125 (Roche Farmacêutica Química Lda, Amadora, Portugal).

SUBJECTS AND METHODS

Study Design

This was a single-center (BIAL's Human Pharmacology Unit, S. Mamede do Coronado, Portugal), Phase I, double-blind, randomized, placebo-controlled, 4-way crossover study with 4 consecutive single-dose treatment periods. The washout period between doses was ≥ 5 days. Because the expected $t_{1/2}$ of nebicapone is 2 to 4 hours,¹³ a washout period of ≥ 5 days is likely to avoid any carryover effect. During the different treatment periods, subjects received a single dose of controlled-release levodopa 100 mg/benserazide 25 mg concomitantly with nebicapone 50, 100, and 200 mg or placebo. At admission to the first period, subjects were sequentially assigned, after confirmation of eligibility, to a randomization number that defined the respective treatment sequence (ie, the order in which the different doses of nebicapone and placebo were administered).

A 50-mg tablet strength of nebicapone[†] and matched placebo tablets were used in the study. Single doses were prepared as follows: nebicapone 50-mg dose = 1 tablet of 50 mg plus 3 tablets of placebo; nebicapone 100-mg dose = 2 tablets of 50 mg plus 2 tablets of placebo; nebicapone 200-mg dose = 4 tablets of 50 mg; and placebo = 4 tablets of placebo. Single doses of controlled-release levodopa 100 mg/benserazide 25 mg comprised 1 capsule. On each occasion, the nebicapone/placebo tablet and the controlled-release levodopa 100-mg/benserazide 25-mg capsule were administered simultaneously (orally).

[†]Manufactured by BIAL (Portela & Ca., S.A.), S. Mamede do

Coronado, Portugal.

Subjects

Healthy male and female volunteers aged 18 to 45 years and with a body mass index (BMI) of 19 to 30 kg/m² were screened for eligibility within 28 days and again within 7 days before first admission. The screening consisted of a medical history; physical examination; recording of vital signs; complete neurologic examination (motor, sensory, reflexes, and coordination examination; mental status; cranial nerve testing; and evaluation of gait and station); 12-lead ECG; hematology, coagulation, plasma biochemistry, and urinalysis tests; HIV, hepatitis B, and hepatitis C serology tests; testing for drugs of abuse and alcohol; urine pregnancy test in women of childbearing potential; and review of the inclusion/exclusion criteria.

An independent ethics committee reviewed and approved the study protocol and the subject information. Written informed consent was obtained from each volunteer before enrollment in the study and before any study-related procedure. The study was conducted according to the Good Clinical Practice recommendations (CPMP/ICH/135/95, finalized on January 17, 1997) and the principles of the Declaration of Helsinki (adopted by the 18th World Medical Association [WMA] General Assembly, Helsinki, Finland, June 1964, and amended by the 55th WMA General Assembly, Tokyo, Japan, 2004). The European Clinical Trials Database registration number is 2005-000648-94.

Assessment Procedures

In each treatment period, eligible subjects were admitted to the Human Pharmacology Unit on the day before receiving study medication for the following assessments: recording of vital signs; medical history and physical examination updates; 12-lead ECG; hematology and plasma biochemistry tests; testing for drugs of abuse and alcohol; and urine pregnancy test in women of childbearing potential. On first admission, subjects were reviewed based on the inclusion/exclusion criteria and were randomly assigned to one of the treatment sequences. On the morning of the dosing day, subjects received a dose of nebicapone/placebo concomitantly with a dose of controlled-release levodopa 100 mg/benserazide 25 mg in fasting conditions (at least 8 hours) and remained in the research facilities until at least 24 hours postdose; they

then left and returned for the next period or the follow-up visit. At given time points between predose and discharge, subjects underwent recording of vital signs, brief neurologic examination, 12-lead ECG, and blood sampling for plasma drug determination and S-COMT activity assay. At discharge, vital signs and ECG were recorded, and hematology and plasma biochemistry tests were performed. Blood pressure was measured in a standing position after 5 minutes of rest. Two measures were conducted 1 minute apart, and the reported result was the mean of these 2 measures. Blood samples (7 mL) for determination of plasma concentrations of levodopa, 3-OMD, and nebicapone, as well as for assay of S-COMT activity, were collected in potassium EDTA Vacutainers at the following times: predose and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, and 24 hours postdose.

Clinical adverse events (AEs) were spontaneously reported by subjects and were monitored throughout the study. Their severity (intensity) was categorized according to a 3-point scale (mild, moderate, and severe), and the causality (potential relationship to drug) was assessed by the investigator before breaking the blinding. No concomitant medication was allowed during the study, except if required for treatment of AEs. A standard diet was served, and alcohol, caffeine, and grapefruit-containing beverages were prohibited.

A follow-up visit was conducted ~7 to 10 days after discharge of the last treatment period or early discontinuation. The following was recorded at this visit: medical history and physical examination updates; recording of vital signs; 12-lead ECG; hematology, plasma biochemistry, and urinalysis tests; and pregnancy test in women of childbearing potential.

Analytical Methods

Blood samples were drawn by direct venipuncture or via an IV catheter into potassium EDTA Vacutainers and centrifuged immediately at ~1500g for 10 minutes at 4°C. The resulting plasma was separated and the aliquots were stored at -70°C until required for analysis. Plasma concentrations of nebicapone, levodopa, and 3-OMD were assayed by the BIAL Laboratory of Pharmacological Research, using validated methods.^{14,15,17} Plasma concentrations of levodopa and 3-OMD were determined using reverse-phase HPLC with electrochemical detection; the lower limit of quantification was 50 ng/mL. Briefly, for the levodopa and 3-OMD determinations, samples were

allowed to thaw at room temperature with sodium metabisulfite 20% (w/v) in a proportion of 25 µL to 1 mL of plasma specimen. Deproteinization was accomplished by adding 307.5 µL of plasma specimen with metabisulfite 20% (w/v) to 200 µL of perchloric acid 1 M followed by centrifugation and filtration. Aliquots of 50 µL were injected in the chromatograph, and sample analysis was performed over a range of 50 to 1000 ng/mL.

Plasma concentrations of nebicapone were determined using an HPLC method with mass spectrophotometer detection. The lower limit of quantification was 50 ng/mL. For the nebicapone determination, samples were allowed to thaw at room temperature and 500 µL were transferred to glass vials for extraction using an automated solid-phase extraction technique (Oasis HLB extraction cartridges, 30 mg, 1 mL, Waters Corporation, Milford, Massachusetts). Aliquots of 5 µL were injected in the chromatograph, and sample analysis was performed over a range of 50 to 2500 ng/mL.

The same blood samples taken for pharmacokinetic assessments were used for the preparation of washed erythrocytes for the assay of S-COMT activity. After collection, the blood samples were immediately centrifuged at ~1500g for 10 minutes at 4°C, and the resulting plasma and uppermost cell layer were removed. The tubes were placed on ice and a volume of cold 0.9% sodium chloride solution equal to double that of the cells was added. The cells were mixed, centrifuged, and washed 3 times using this procedure. Washed cells (500 µL) in 2-mL micro tubes were stored at -70°C until required for analysis. S-COMT activity was determined, using previously validated methods described elsewhere,^{14,15,18,19} by the BIAL Laboratory of Pharmacological Research. S-COMT activity was assayed by means of HPLC with electrochemical detection and expressed as the amount (in picomoles) of metanephrine formed by the action of S-COMT on an epinephrine substrate (per milligram of hemoglobin per hour).

Pharmacokinetic and Pharmacodynamic Parameters

The following parameters for levodopa, 3-OMD, and nebicapone were derived by noncompartmental analysis from the concentration–time profiles: C_{max} ; T_{max} ; AUC_{0-t} , calculated by the linear trapezoidal rule; $AUC_{0-\infty}$, calculated from $AUC_{0-t} + (C_{last}/\lambda_z)$, where

C_{last} is the last quantifiable concentration; apparent terminal rate constant (λ_z), calculated by log-linear regression of the terminal segment of the drug plasma concentration–time curve; and $t_{1/2}$, calculated by $\ln 2 / \lambda_z$.²⁰

The following mean pharmacodynamic parameters of S-COMT activity were derived from the individual S-COMT activity profiles: maximum inhibition of S-COMT activity (E_{max}), time to occurrence of E_{max} (T_{Emax}), and area under the effect–time curve (AUEC). The predose value was taken as baseline value. Point estimates (PEs) and 90% CIs for the geometric mean ratios of E_{max} and AUEC were calculated.

The pharmacokinetic and pharmacodynamic parameters were derived using WinNonlin version 4.1 (Pharsight Corporation, Mountain View, California).

Tolerability

Individual and summary blood pressure, heart rate, ECG parameters, and clinical laboratory data were presented in tabular form with mean, median, SD, and range (minimum and maximum) as appropriate. For the laboratory tolerability data, out-of-range values were flagged in the data listings, and clinically significant abnormalities were reported as AEs. AEs were tabulated and summarized according to version 9.0 of the Medical Dictionary for Regulatory Activities (MedDRA MSSO, Reston, Virginia).

Statistical Analysis

Summary statistics on the pharmacokinetic parameters were reported, as appropriate, using the geometric mean, arithmetic mean, SD, %CV, median, minimum, and maximum. The main levodopa and 3-OMD pharmacokinetic parameters (C_{max} and AUC_{0-t}) were compared between treatment groups using an ANOVA with sequence, subject (sequence), treatment, and period effects after logarithmic transformation of the data. According to the protocol, PEs and 90% CIs for the test/reference geometric mean ratios of C_{max} and $AUC_{0-\infty}$ of levodopa and 3-OMD after administration of controlled-release levodopa 100 mg/benserazide 25 mg concomitantly with nebicapone versus after administration of controlled-release levodopa 100 mg/benserazide 25 mg concomitantly with placebo were to be calculated; however, levodopa $AUC_{0-\infty}$ could not be reliably calculated in the placebo period, and AUC_{0-t} was therefore used to determine the levodopa AUC ratio.

Statistical significance was concluded when the 90% CI excluded the unit.²¹

Nebicapone's effectiveness depends on the increase of levodopa systemic exposure,³ and the AUC of levodopa can be considered the primary pharmacokinetic parameter. Assuming an α error of 5% (2-tailed) and an approximate intrasubject %CV of 20% for AUC, 16 subjects would provide a 80% probability (power) that the 90% CI for the test/reference AUC geometric means ratio (PE) after nebicapone (test) and placebo (reference) administered concomitantly with controlled-release levodopa 100 mg/benserazide 25 mg falls within the acceptance interval of 80% to 125%.

The statistical computations were performed using SAS Release 8.2 or higher (SAS Institute Inc., Cary, North Carolina).

RESULTS

A total of 17 healthy young subjects were enrolled. One subject withdrew his consent after the first treatment period and was replaced. The replacement subject was allocated to the same treatment sequence and completed all 4 treatment periods. Sixteen subjects (8 females, 8 males; mean [SD] age, 26.13 [6.29] years; median age, 23.5 years; age range, 19–37 years; weight, 69.4 [12.4] kg; median weight, 64.5 kg; weight range, 51–96 kg; BMI, 24.0 [3.0] kg/m²; median BMI, 24.0 kg/m²; BMI range, 20–30 kg/m²) completed the 4 treatment periods and were available for pharmacokinetic and pharmacodynamic analyses.

Pharmacokinetics

Levodopa and 3-O-Methyldopa

Mean plasma concentration–time profiles of levodopa and 3-OMD after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg concomitantly with nebicapone 50, 100, and 200 mg or placebo are displayed in [Figures 1](#) and [2](#), respectively. [Table I](#) presents the main pharmacokinetic parameters. Mean levodopa C_{max} values were attained at 1.8 to 3.0 hours (median T_{max}) postdose. Thereafter, plasma levodopa concentrations declined, with a mean $t_{1/2}$ of ~2 hours.

[Table II](#) depicts the test/reference PEs and 90% CIs of the levodopa and 3-OMD main pharmacokinetic parameters. Extent of exposure to levodopa, as assessed by AUC_{0-t} , increased with all doses of nebicapone in relation to placebo, but the difference did not

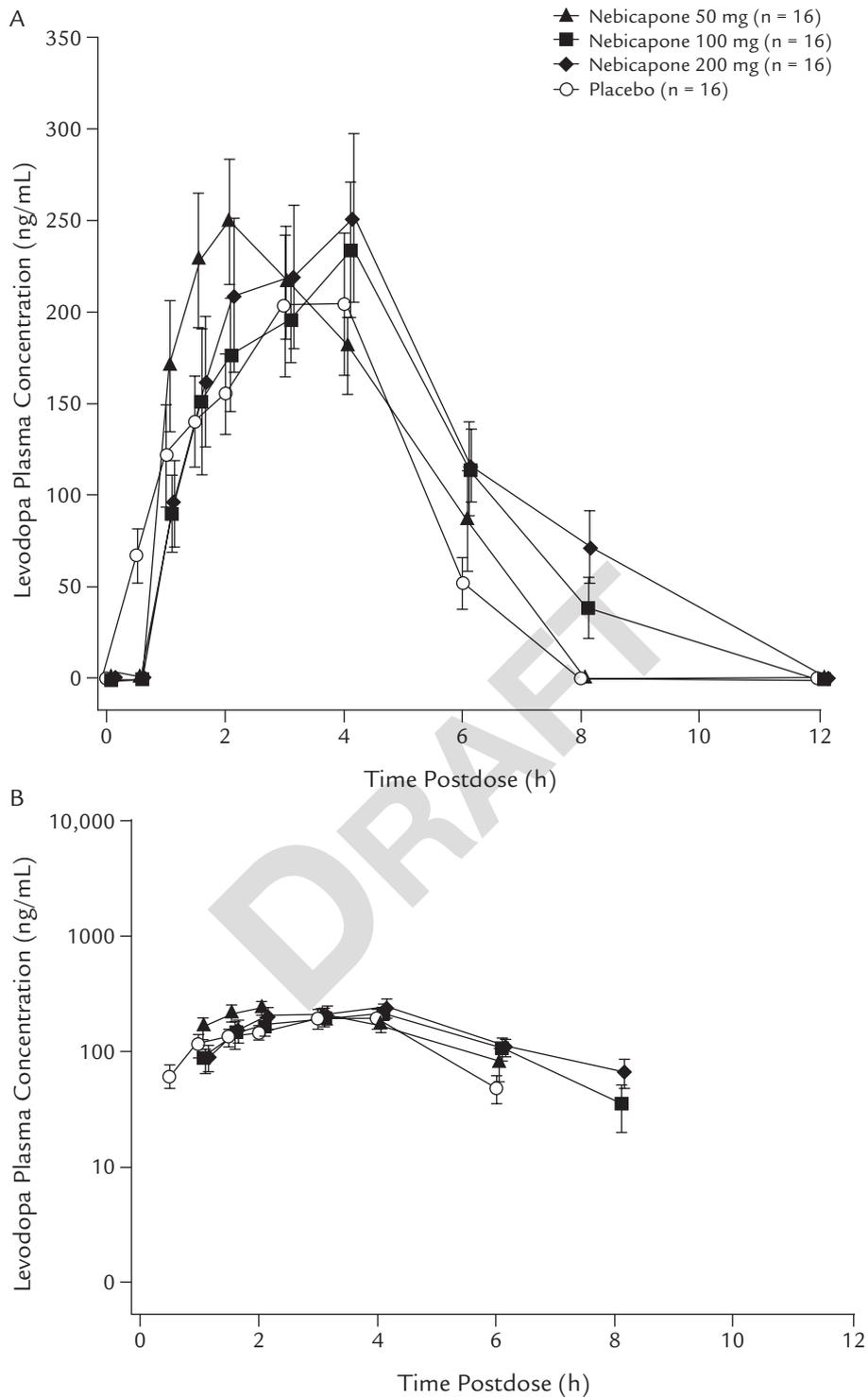


Figure 1. Mean (SEM) plasma concentration–time profiles of levodopa after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg (Trademark: Madopar® HBS 125 [Roche Farmacêutica Química Lda, Amadora, Portugal]) administered concomitantly with nebicapone or placebo (n = 16 per dose). (A) Linear scale; (B) semi-log scale.

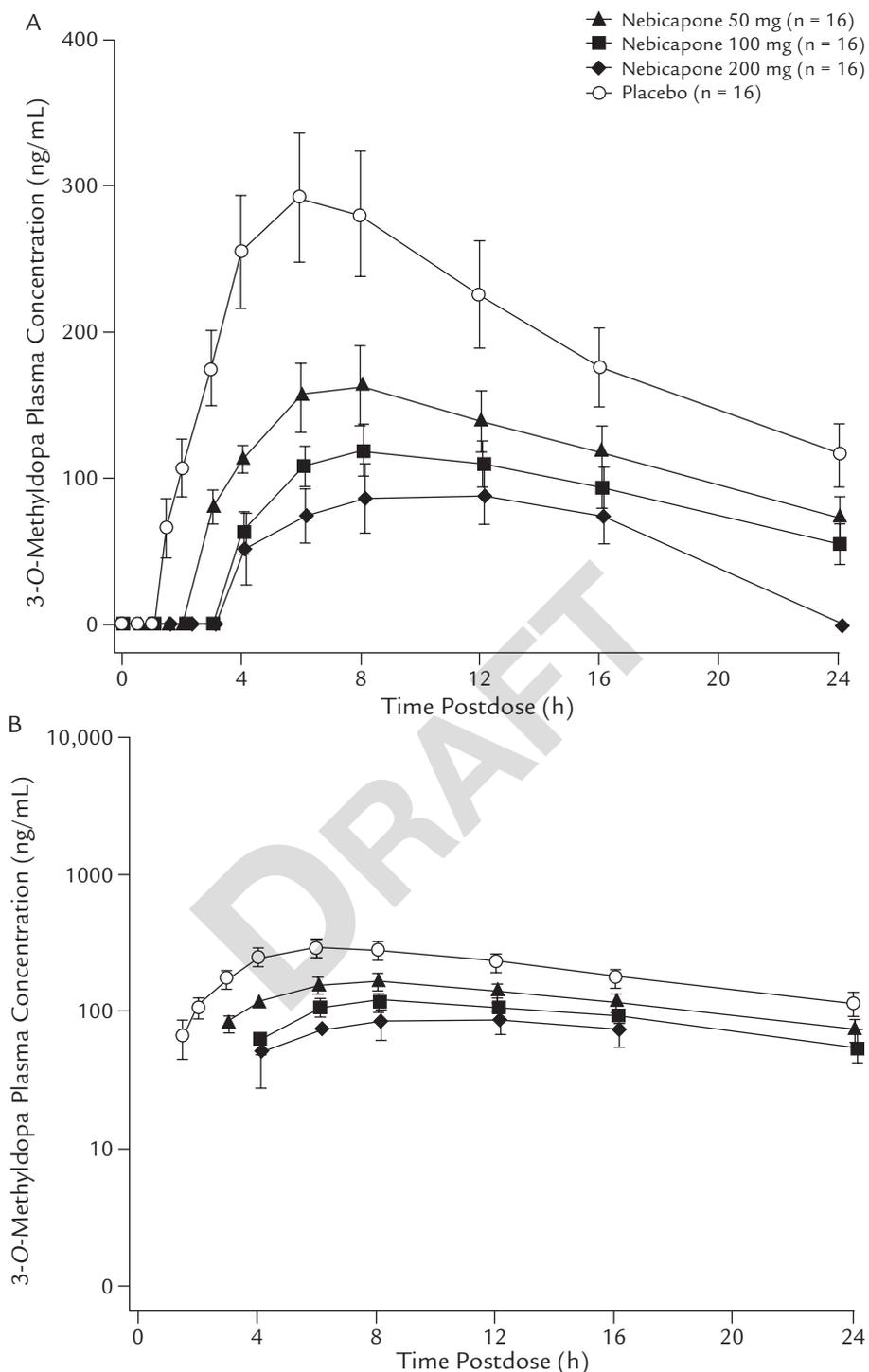


Figure 2. Mean (SEM) plasma concentration–time profiles of the levodopa 3-*O*-methylated metabolite (3-*O*-methyldopa) after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg (Trademark: Madopar® HBS 125 [Roche Farmacêutica Química Lda, Amadora, Portugal]) administered concomitantly with nebicapone or placebo ($n = 16$ per dose). (A) Linear scale; (B) semi-log scale. There is no 24-hour point at the highest dose of “B” because the concentration was zero.

Table I. Mean (%CV) weight-adjusted pharmacokinetic parameters of levodopa and the levodopa 3-*O*-methylated metabolite (3-*O*-methyldopa) after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg* administered concomitantly with nebicapone or placebo (n = 16 per dose).

Dose	C_{max} , (ng/mL · kg)	T_{max} , h [†]	AUC_{0-t} , (ng·h/mL · kg)	$t_{1/2}$, h
Levodopa				
Nebicapone 50 mg	5.0 (53)	1.8 (1.0–6.0)	15.7 (59)	NRA
Nebicapone 100 mg	5.0 (58)	3.0 (1.0–6.0)	15.8 (60)	NRA
Nebicapone 200 mg	5.3 (56)	2.5 (1.0–8.0)	18.4 (62)	NRA
Placebo	4.0 (62)	3.0 (0.5–4.0)	13.1 (78)	NRA
3-<i>O</i>-Methyldopa				
Nebicapone 50 mg	2.6 (68)	6.0 (2.0–8.0)	39.9 (65)	NRA
Nebicapone 100 mg	2.0 (55)	8.0 (3.0–12.0)	31.2 (60)	NRA
Nebicapone 200 mg	2.1 (66)	8.0 (3.0–12.0)	29.7 (70)	NRA
Placebo	5.0 (62)	6.0 (4.0–8.0)	76.1 (65)	NRA

NRA = could not be reliably assessed because not enough data points were available during the terminal monoexponential phase for several subjects.

*Trademark: Madopar[®] HBS 125 (Roche Farmacêutica Química Lda, Amadora, Portugal).

[†]Values given as median (range).

attain statistical significance, which may be related to a relatively high intersubject variability: %CVs ranged from 48.0% with nebicapone 100 mg to 66.8% with placebo. Peak exposure to levodopa, as assessed by C_{max} , was significantly higher after any dose of neb-

icapone compared with placebo. No statistically significant differences were found between levodopa T_{max} values when nebicapone 50, 100, and 200 mg were compared with placebo.

Exposure to 3-OMD, as reflected by C_{max} and

Table II. Point estimates (90% CIs) of the levodopa and the levodopa 3-*O*-methylated metabolite (3-*O*-methyldopa) pharmacokinetic parameters obtained after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg* administered concomitantly with placebo or nebicapone (n = 16 per dose).

Nebicapone Dose/Placebo	C_{max}	AUC_{0-t}
Levodopa		
Nebicapone 50 mg/placebo	125 (101–152) [†]	114 (83–165)
Nebicapone 100 mg/placebo	130 (106–160) [†]	137 (91–180)
Nebicapone 200 mg/placebo	134 (109–164) [†]	142 (97–192)
3-<i>O</i>-methyldopa		
Nebicapone 50 mg/placebo	56 (45–69) [†]	67 (53–78) [†]
Nebicapone 100 mg/placebo	43 (35–54) [†]	63 (51–77) [†]
Nebicapone 200 mg/placebo	42 (34–53) [†]	55 (49–75) [†]

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[†]Statistically significant difference (the 90% CIs for geometric mean ratios did not include the unit).

$AUC_{0-\infty}$, significantly and dose dependently decreased with all doses of nebicapone compared with placebo. After administration of nebicapone 50, 100, and 200 mg, 3-OMD C_{max} decreased 44%, 57%, and 58%, and $AUC_{0-\infty}$ decreased 33%, 37%, and 45%, respectively, compared with placebo. No statistically significant differences were found between 3-OMD T_{max} values when nebicapone 50, 100, and 200 mg were compared with placebo.

Nebicapone

Mean nebicapone plasma concentration–time profiles and pharmacokinetic parameters are presented in **Figure 3** and **Table III**, respectively. After oral administration of single doses of nebicapone 50, 100, and 200 mg concomitantly with a single dose of controlled-release levodopa 100 mg/benserazide 25 mg, nebicapone T_{max} occurred at ~1 hour postdose. Findings for $t_{1/2}$ could not be reliably assessed because not enough data points were available during the terminal monoexponential phase in several subjects. Systemic exposure to nebicapone was approximately dose proportional: for a dose increase in the ratio of 1:2:2 (50:100:200 mg), C_{max} increased in the ratio of 1:1.7:2.1 (35.0, 58.6, and 129.0 ng/mL) and $AUC_{0-\infty}$ increased in the ratio of 1:2.1:2.2 (60.1, 128.0, and 285.0 ng · h/mL)

Pharmacodynamics

Mean S-COMT activity profiles from baseline (pre-dose) after oral administration of single doses of controlled-release levodopa 100 mg/benserazide 25 mg concomitantly with nebicapone 50, 100, and 200 mg or placebo are presented in **Figure 4** and **Table IV**. After oral administration of controlled-release levodopa 100 mg/benserazide 25 mg and nebicapone, maximum S-COMT inhibition (E_{max}) occurred between 1.4 and 1.5 hours postdose (T_{Emax}) and was dose dependent. It ranged from 57% with nebicapone 50 mg to 74% with nebicapone 200 mg. In relation to placebo, peak S-COMT activity decreased 54% after nebicapone 50 mg, 58% after nebicapone 100 mg, and 72% after nebicapone 200 mg. The time to return to baseline of enzyme activity ranged from 6 hours with nebicapone 50 mg to 12 hours with nebicapone 200 mg, and followed the same dose-dependent tendency observed with nebicapone-induced E_{max} changes. **Table V** depicts the test/reference PEs and 90% CIs of S-COMT's main pharmacodynamic parameters.

Tolerability

Ten (58.8%) of 17 subjects who participated in the study reported a total of 19 AEs. Eight of these AEs were assessed by the investigator as possibly related to treatments and 11 AEs as not related. All AEs were mild in severity. The most frequent AEs were pharyngitis (3 cases, reported by 3 subjects), increased creatinine phosphokinase (2 cases, reported by 2 subjects), frontal headache (2 cases, reported by 2 subjects), and orthostatic dizziness (2 cases, reported by 2 subjects). By treatment period, AEs possibly related to treatment were distributed as follows: 2 AEs (reported by 2 subjects) with placebo, 1 AE (reported by 1 subject) with nebicapone 50 mg, and 5 AEs (reported by 4 subjects) with nebicapone 100 mg. There were no AEs reported as possibly related to treatment in the nebicapone 200-mg treatment period.

There were no serious AEs or discontinuations due to AEs. Overall, no clinically relevant drug-related abnormalities were found in the results from the 12-lead ECG recordings, vital signs, or clinical laboratory tolerability tests (including liver function tests). One subject withdrew his informed consent 2 days after discharge of his first treatment period (nebicapone 200 mg).

DISCUSSION

Single-dose interaction studies between nebicapone and immediate-release levodopa/carbidopa¹⁴ and immediate-release levodopa 100 mg/benserazide 25 mg¹⁵ in healthy subjects reported that doses of nebicapone 50, 100, and 200 mg increase systemic exposure to levodopa. In the present study, the coadministration of single doses of nebicapone 50, 100, and 200 mg with a single dose of controlled-release levodopa 100 mg/benserazide 25 mg was found to dose dependently and significantly inhibit S-COMT activity and to reduce the formation of 3-OMD in these healthy volunteers. Levodopa bioavailability was not significantly affected.

The primary end point was the effect of nebicapone on levodopa systemic exposure. Besides a relatively high %CV in the levodopa pharmacokinetic parameters, analyses were complicated by difficulties in reliably estimating $AUC_{0-\infty}$ and $t_{1/2}$, as well as the impossibility of calculating $AUC_{0-\infty}$ geometric mean ratios. Therefore, extent of exposure was assessed using AUC_{0-t} .

Extent of exposure to levodopa, increased numeri-

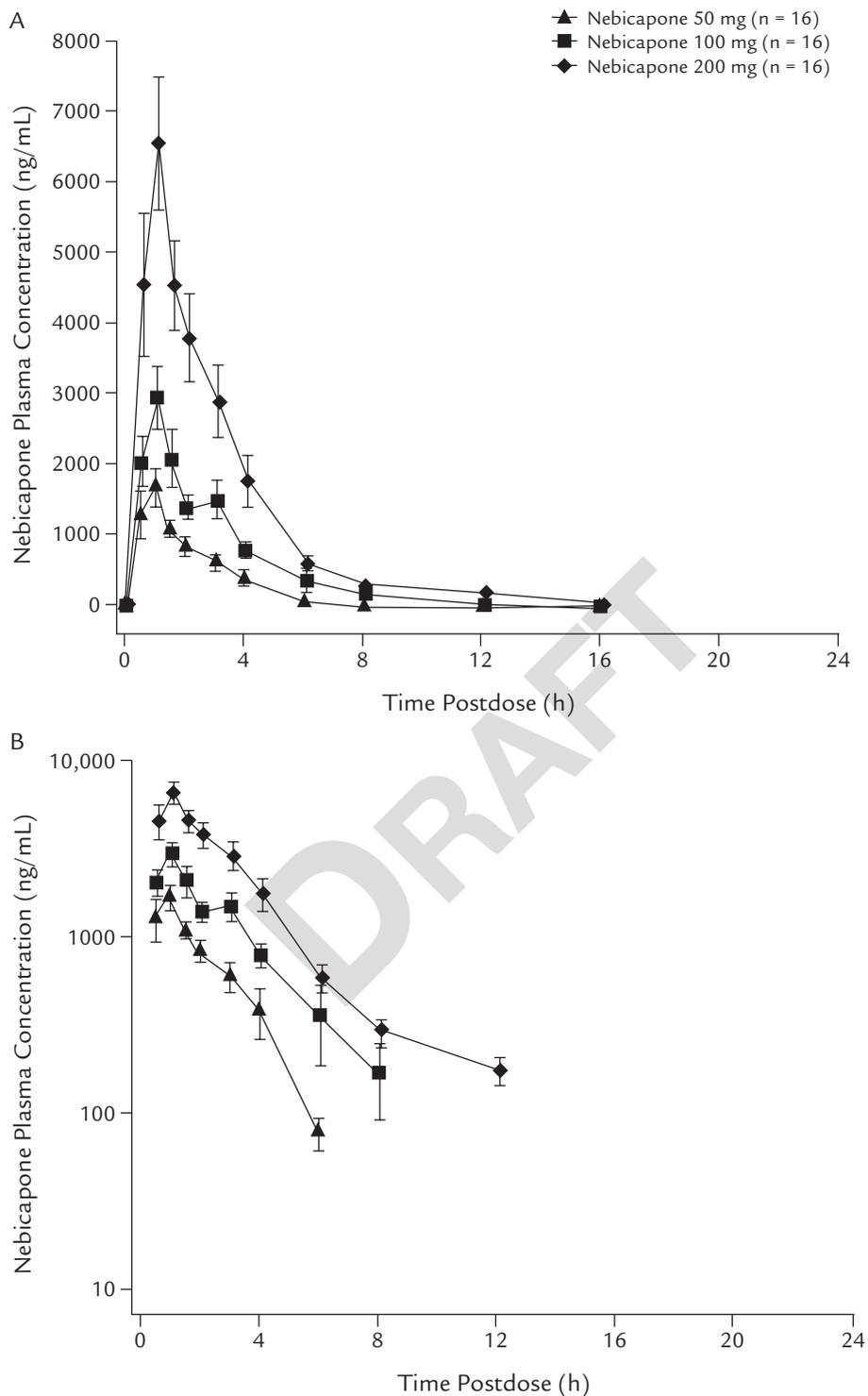


Figure 3. Mean (SEM) nebicapone plasma concentration–time profiles after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg (Trademark: Madopar® HBS 125 [Roche Farmacêutica Química Lda, Amadora, Portugal]) administered concomitantly with nebicapone (n = 16 per dose). (A) Linear scale; (B) semi-log scale.

Table III. Mean (%CV) weight-adjusted pharmacokinetic parameters of nebicapone after coadministration of a single dose of controlled-release levodopa 100 mg/benserazide 25 mg* and nebicapone 50, 100, and 200 mg (n = 16 per dose).

Dose	C_{max} , (ng/mL · kg)	T_{max} , h [†]	AUC_{0-t} , (ng · h/mL · kg)	$t_{1/2}$, h
Nebicapone 50 mg	35.0 (43)	1.0 (0.5–4.0)	60.1 (40)	NRA
Nebicapone 100 mg	58.6 (35)	1.0 (0.5–6.0)	128.0 (38)	NRA
Nebicapone 200 mg	129.0 (43)	1.0 (0.5–4.0)	285.0 (39)	NRA

NRA = could not be reliably assessable, because not enough data points were available during the terminal monoexponential phase for several subjects.

*Trademark: Madopar[®] HBS 125 (Roche Farmacêutica Química Lda, Amadora, Portugal).

[†]Values are given as median (range).

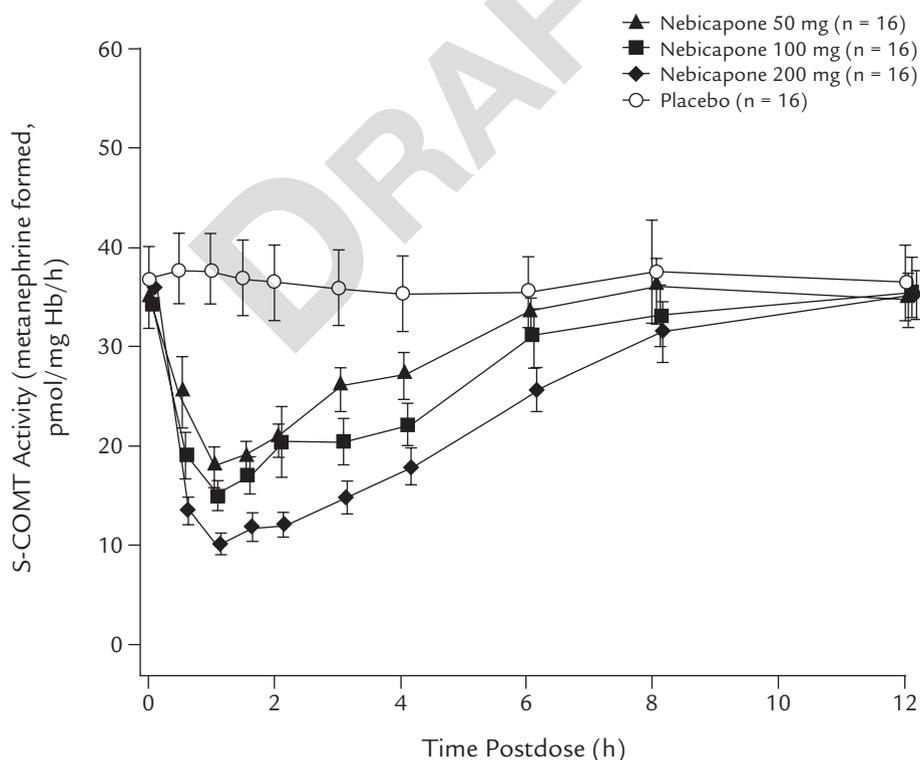


Figure 4. Mean (SEM) erythrocyte-soluble catechol-O-methyltransferase (S-COMT) activity profile from baseline (predose) after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg (Trademark: Madopar[®] HBS 125 [Roche Farmacêutica Química Lda, Amadora, Portugal]) administered concomitantly with nebicapone or placebo (n = 16 per dose). Hb = hemoglobin.

Table IV. Mean (SD) erythrocyte-soluble catechol-*O*-methyltransferase (S-COMT) pharmacodynamic parameters after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg* administered concomitantly with nebicapone or placebo (n = 16 per dose).

Dose	E_0 , pmol/mg Hb/h	E_{max} , pmol/mg Hb/h	T_{Emax} , h	$([E_0 - E_{max}]/E_0) \times 100$, %	AUEC, pmol/mg Hb/h
Nebicapone 50 mg	35.1 (13.3)	14.3 (6.0)	1.4 (0.9)	56.8 (13.3)	799 (224)
Nebicapone 100 mg	34.3 (13.6)	12.9 (4.8)	1.4 (0.8)	61.4 (8.6)	767 (285)
Nebicapone 200 mg	35.9 (12.0)	8.3 (2.4)	1.5 (1.0)	74.3 (12.4)	747 (219)
Placebo	36.9 (12.9)	31.4 (13.9)	5.5 (4.1)	15.8 (15.7)	875 (352)

E_0 = predose value taken as the baseline value; Hb = hemoglobin; E_{max} = maximum inhibition of S-COMT activity; T_{Emax} = time to occurrence of E_{max} ; AUEC = area under the effect-time curve.

*Trademark: Madopar® HBS 125 (Roche Farmacêutica Química Lda, Amadora, Portugal).

cally with all doses of nebicapone compared with placebo but did not reach statistical significance. The nonsignificant increase was 14% with nebicapone 50 mg, 37% with nebicapone 100 mg, and 42% with nebicapone 200 mg. However, there was a relatively high variability in subjects' exposure to levodopa, likely related to the levodopa/benserazide formulation because it occurred both in the placebo periods and the nebicapone treatment periods. Peak exposure to levodopa, as assessed by C_{max} , was significantly higher after any dose of nebicapone compared with placebo: there was an increase of 25% with nebicapone 50 mg, 30% with nebicapone 100 mg, and 34% with nebicapone 200 mg. No statistically significant differences

were found between levodopa T_{max} values when nebicapone 50, 100, and 200 mg were compared with placebo.

In a study in 16 healthy male subjects, repeated doses of entacapone 200 mg administered 4 times daily at 4-hour intervals concomitantly with a single dose of controlled-release levodopa 100 mg/carbidopa 25 mg induced an increase of 39% in levodopa AUC and a decrease of 50% in 3-OMD AUC.²² In an open-label randomized study in which single doses of 100- to 800-mg entacapone were administered with controlled-release levodopa/carbidopa to 12 healthy male subjects, the highest increase in AUC was 33% and occurred with entacapone 400 mg.²³ In a study in

Table V. Point estimates (90% CIs) of catechol-*O*-methyltransferase (COMT) pharmacodynamic parameters obtained after a single dose of controlled-release levodopa 100 mg/benserazide 25 mg* administered concomitantly with nebicapone or placebo (n = 16 per dose).

Dose	E_{max}	AUEC
Nebicapone 50 mg/placebo	47 (39–56) [†]	95 (88–102)
Nebicapone 100 mg/placebo	42 (35–50) [†]	89 (82–96) [†]
Nebicapone 200 mg/placebo	28 (23–33) [†]	88 (82–95) [†]

E_{max} = maximum inhibition of erythrocyte-soluble COMT activity; AUEC = area under the effect-time curve.

*Trademark: Madopar® HBS 125 (Roche Farmacêutica Química Lda, Amadora, Portugal).

[†]Statistically significant difference (the 90% CIs for the geometric mean ratios did not include the unit).

healthy subjects in which a single dose of tolcapone 200 mg was coadministered with a single dose of controlled-release levodopa 200 mg/carbidopa 50 mg, levodopa AUC increased by 80% and 3-OMD AUC decreased by 70%.²⁴

In the present study, after administration of nebicapone 50, 100, and 200 mg, maximum S-COMT inhibition occurred between 1.4 and 1.5 hours postdose ($T_{E_{max}}$) and was dose dependent. In relation to placebo, peak S-COMT activity decreased 54% after nebicapone 50 mg, 58% after nebicapone 100 mg, and 72% after nebicapone 200 mg.

The exposure to 3-OMD, when considering C_{max} and $AUC_{0-\infty}$, significantly decreased with all doses of nebicapone in relation to placebo. No statistically significant differences were found between 3-OMD T_{max} values when nebicapone 50, 100, and 200 mg were compared with placebo.

Pharmacokinetics of nebicapone appeared to be dose proportional. A second peak in the nebicapone plasma concentration–time profile was noted at ~4 hours postdose, which may be due to enterohepatic circulation. All treatments tested in this study were generally well tolerated. All AEs were mild in severity, and no serious AEs or discontinuations due to AEs were reported. No clinically relevant abnormalities were found in clinical laboratory tolerability examinations, vital signs, or ECG recordings.

Limitations

The study did have some limitations. It used a single-dose approach and enrolled only healthy subjects under controlled conditions. Thus, the clinical significance of the findings—namely, in preventing motor fluctuations in levodopa-treated PD patients—must be clarified in additional studies in patients with the target disorder.

CONCLUSIONS

In this study in healthy adult volunteers, single doses of nebicapone 50, 100, and 200 mg administered concomitantly with a single dose of controlled-release levodopa 100 mg/benserazide 25 mg appeared to be well tolerated, and dose dependently inhibited S-COMT activity and reduced 3-OMD formation compared with placebo. There was no significant difference in levodopa bioavailability.

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