CMRO
Current Medical Research & Opinion Vol. 26, No. 5, 2010, 1097–1108
0300-7995
doi:10.1185/03007991003694472
Article 5168b/469969

Original article

Chronopharmacology of nebicapone, a new catechol-*O*-methyltransferase inhibitor

Luis Almeida
Department of Research and Development, Bial – Portela & C[a] SA, S. Mamede do Coronado, Portugal
Institute of Pharmacology and Therapeutics, Faculty of Medicine, University of Oporto, Portugal
Health Sciences Section, University of Aveiro, Portugal, Portugal

Ana I. Loureiro
Manuel Vaz-da-Silva
Leonel Torrão
Joana Maia
Carlos Fernandes-Lopes
Department of Research and Development, Bial – Portela & C[a] SA, S. Mamede do Coronado, Portugal

Amílcar Falcão
4Health Ltd, Cantanhede, Portugal

Bruno Igreja
Lyndon Wright
Department of Research and Development, Bial – Portela & C[a] SA, S. Mamede do Coronado, Portugal

Patricio Soares-da-Silva
Institute of Pharmacology and Therapeutics, Faculty of Medicine, University of Oporto, Portugal
Department of Research and Development, Bial – Portela & C[a] SA, S. Mamede do Coronado, Portugal

Address for correspondence:
Professor P. Soares-da-Silva, Department of Research & Development, Bial – Portela & C[a] SA, À Avenida da Siderurgia Nacional, 4745-457 S. Mamede do Coronado, Portugal.
Tel.: +351-22-9866100; Fax: +351-22-9866192; psoares.silva@bial.com

Key-words:
Catechol-*O*-methyltransferase inhibitors – Chronopharmacokinetics – Chronopharmacology – Nebicapone – Parkinson's disease

Accepted: 11 February 2010; published online: 12 March 2010
Citation: Curr Med Res Opin 2010; 26:1097–1108

Abstract

Objective:
To investigate the chronopharmacology of nebicapone, a new catechol-*O*-methyltransferase (COMT) inhibitor currently being developed for use as an adjunct to levodopa/carbidopa or levodopa/benserazide in the treatment of Parkinson's disease.

Methods:
This was a double-blind, randomised, placebo-controlled, parallel-group study. Eighteen Caucasian subjects were randomly assigned to treatment with either nebicapone 100 mg ($n=6$), nebicapone 200 mg ($n=6$) or placebo ($n=6$) at 4-h intervals for 7 days. First dose occurred at 8:00 AM on day 1 and last dose at 8:00 AM on day 8. Blood samples for the determination of plasma drug concentrations of nebicapone and its glucuronidated and methylated metabolites and for the assay of erythrocyte soluble COMT (S-COMT) activity were taken at frequent times following the first and last doses, and before the 8:00 AM and 8:00 PM doses on days 2–7.

Results:
Three men and three women in each group participated in the study. Mean $\pm$ SD (range) age of study participants was 23.7 ± 3.1 (21–28) years in the nebicapone 100 mg group, 22.2 ± 0.4 (22–23) years in the nebicapone 200 mg group and 24.3 ± 5.4 (18–32) in the placebo group. A circadian variation in the pre-dose nebicapone and nebicapone-glucuronide plasma concentrations was apparent. Both nebicapone and nebicapone-glucuronide levels were lower before the 8 PM dose in comparison to the 8 AM dose, suggesting that the absorption of nebicapone may follow a circadian variation. S-COMT activity showed no circadian variation in the placebo group. Therefore, the S-COMT activity variation found in nebicapone-treated subjects is considered to be due to changes in plasma concentrations of nebicapone, which is consistent with the fact that the pre-dose S-COMT activity was lower at the time at which nebicapone levels were maximal. Four subjects in the nebicapone 100 mg and placebo groups and six subjects in the nebicapone 200 mg group reported at least one adverse event (AE). All AEs were of mild or moderate intensity. Both nebicapone treatment regimens were subjectively well-tolerated, but a clinically relevant elevation in aspartate transaminase was observed in one subject of each nebicapone group.

Conclusion:
Nebicapone showed chronopharmacology in young Caucasian healthy subjects. The clinical impact of the circadian variation in the nebicapone metabolism and activity in Parkinson's disease patients deserves evaluation as it may have implications for drug prescription by modulating the distribution of the total daily dose along the 24-h scale.

Introduction

Nebicapone (BIA 3-202) is a new catechol-O-methyltransferase (COMT) inhibitor in development as an adjunct to levodopa/benserazide or levodopa/carbidopa in the treatment of patients suffering from Parkinson's disease (PD).

Nebicapone has shown to be generally well-tolerated in studies in healthy subjects[1–7] and in PD patients[8]. Nebicapone in doses of 100 mg and 200 mg administered to healthy subjects[3,4] and in doses of 75 mg and 150 mg administered to patients[8] was found to effectively inhibit COMT and increase levodopa exposure following co-administration with levodopa/benserazide or levodopa/carbidopa.

Nebicapone is rapidly absorbed, with a t_{max} usually ranging between 1 and 3 h[1,2]. Pharmacokinetics of nebicapone was unaffected by the presence of food[1]. *In vitro* binding of [^{14}C] nebicapone to plasma proteins was high (>99%) with no evidence of concentration-dependent binding. Binding of [^{14}C] nebicapone to blood cells is low (10–30%) with no evidence of concentration dependent binding. Time to maximum observed concentration (t_{max}) of nebicapone tended to occur earlier when administered concomitantly with levodopa/carbidopa to young subjects than when administered alone to elderly patients[8]; however, age did not affect nebicapone maximum observed concentration (C_{max}). In humans, glucuronidation by UDP-glucuronosyltransferases and 3-*O*-methylation by COMT appear to be the major metabolic pathways of nebicapone, leading to the formation of nebicapone-glucuronide and 3-*O*-methyl-nebicapone, respectively[9,10]. Several other metabolites were found to occur in minor amounts and correspond to sulphated, reduced, and sulphated plus reduced derivatives of nebicapone[9].

The absorption, distribution, metabolism, excretion and therapeutic effect of drugs can be influenced by several physiological functions that can change along the day. Chronopharmacokinetics and chronopharmacodynamics are rarely characterised during the development of new drugs. However, drug chronopharmacology may be clinically relevant as it may have implications for drug prescription by modulating the distribution of the total daily dose along the 24-h scale[11]. In a previous study in healthy volunteers there was a suggestion that nebicapone could present chronopharmacokinetics[2]. The aim of the current study was to further investigate the chronopharmacology of nebicapone in healthy subjects.

Population and methods

Participants

Healthy male or female subjects between the ages of 18 and 45 years, within a body mass index (BMI) range of 19–28 kg/m^2, non-smokers or smokers of less than ten cigarettes per day, were eligible for the study. The healthy status was assessed on basis of a medical history, physical examination, electrocardiogram, and clinical laboratory safety tests (haematology, coagulation, plasma biochemistry, urinalysis, and hepatitis B, hepatitis C and HIV serology).

Study design

This was a single-centre (Human Pharmacology Unit, Bial – Portela & C[a], SA, S. Mamede do Coronado, Portugal), double-blind, randomised, placebo-controlled, parallel-group study. Eighteen subjects were randomly assigned to treatment with either nebicapone 100 mg ($n = 6$), nebicapone 200 mg ($n = 6$) or placebo ($n = 6$) at 4-h intervals (6 times/day), for 7 days. The randomisation code was generated using computerised techniques. Eligible subjects were admitted to the research facilities on the morning of the day prior (day 0) to receiving the first dose of study medication (day 1, 8:00 AM) and remained in the research facilities under continuous clinical supervision until at least 48 h after receiving their final dose (day 8, 8:00 AM). Then, subjects left the research facilities and returned on days 15, 22 and 29 for blood sampling and clinical evaluation. Diet, activities and sleeping time were strictly controlled from admission until leaving the clinical research facilities. Alcohol- and grapefruit-containing food and beverages were prohibited. Meals were served as follows: days 1 and 8 – no breakfast, light snack 2 h after the 8:00 AM dose, lunch 1 h after the 12:00 AM dose, snack 1 h after the 4:00 PM dose, and dinner 1 h after the 8:00 PM dose; days 2–7 – breakfast 1 h after 8:00 AM dose, lunch 1 h after the 12:00 AM dose, snack 1 h after the 4:00 PM dose, and dinner 1 h after the 8:00 PM dose. Nebicapone/placebo doses were administered with 200 mL of water.

Blood samples (7 mL) for the determination of plasma concentrations of nebicapone, nebicapone-glucuronide and 3-*O*-methyl-nebicapone, and for the assay of the erythrocyte COMT activity were taken into potassium EDTA Vacutainers at the following times: day 1: pre-dose, ½, 1, 1½, 2, 3, 4, 6, 8, 12 and 16 h post-first dose; day 8: pre-final dose (8:00 AM), ½, 1, 1½, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 168, 336, and 504 h post dose; days 2, 3, 4, 5, 6, and 7: before the 8:00 AM and 8:00 PM doses. Urine for urinalysis and blood for coagulation, haematology and plasma biochemistry tests were collected at admission, and on days 2, 10 and 29. Blood samples were drawn either by direct venepuncture or via an intravenous catheter. A 24-h urine collection for the assay of urinary electrolytes and creatinine was performed at admission and on days 1 and 9. No medication other than study products was allowed during the study, unless required for treatment of adverse events (AEs).

AEs were monitored throughout the entire study period. An AE was any undesirable event occurring to a subject during the study whether or not considered related to the investigational product. AEs were recorded from

spontaneous reports, indirect inquiry or investigator observation. Clinically relevant abnormalities in laboratory safety tests were reported as AEs. All AEs were assessed by the investigator with regards to intensity (severity), causality and seriousness.

The study was conducted in accordance with the Declaration of Helsinki principles, the Good Clinical Practice guidelines and the local laws. An Independent Ethics Committee reviewed and approved the study. All subjects gave their written informed consent prior participation.

Bioanalytical methods

Determination of nebicapone, nebicapone-glucuronide and 3-O-methyl-nebicapone plasma concentrations

After collection, blood samples were centrifuged at approximately 1500 *g* for 10 min at 4°C. The resulting plasma was separated into four 0.8-mL aliquots and stored at −20°C until required for analysis. Plasma concentrations of nebicapone and its glucuronidated and 3-*O*-metylated metabolites were analysed under Good Laboratory Practice (GLP) conditions by the Laboratory of Pharmacological Research of Bial (Portela & C[a], SA; S. Mamede do Coronado, Portugal) trough a validated method using high performance liquid chromatography with mass spectrometer detection (HPLC-MSD) following solid phase extraction. To 1 mL plasma samples, 1 mL of internal standard (tolcapone, 2.2 µg/ml in 0.1 M phosphate buffer at pH 2) was added. Samples (0.3 mL) were then loaded onto the cartridges (Oasis HLB C18, 30 mg, 1 mL water) preconditioned with 1 mL acetonitrile and washed twice with 1 mL of phosphate buffer 0.1 M, pH 2. Following sample application, the cartridges were washed with 2 × 1 mL 0.1 M phosphate buffer pH 2. After a second wash the cartridges were flushed with an air push of 10 mL at 6 mL/min. The analytes were eluted with 2 × 0.25 mL acetonitrile, 1% formic acid and once with 0.25 mL of water with an air push of 2 mL at 6 mL/min. To the eluted samples 0.2 mL of water, 1% formic acid were added and mixed twice with aspirating dispensing cycles. The eluted samples were injected into HPLC-MSD modular system Series 1100 from Agilent. Separation was achieved using a Zorbax SB-18, 3 µm 30 × 4.6 mm column. Mobile phase A consisted of acetonitrile : formic acid (99:1, v.v.) and mobile phase B consisted of water : formic acid (99:1, v.v.). The flow rate was 0.5 mL/min. A calibration curve, over the nominal concentration range of 1043–8000 ng/mL for nebicapone, 2609–4000 ng/mL for nebicapone-glucuronide, and 261–4000 ng/mL for 3-*O*-methyl-nebicapone, and a set of quality control (QC) samples, two at each of three concentrations, were run with each batch of study samples. The QC samples were used to monitor the performance of the method and two batches were required to complete the sample analyses. The data for the plasma QC samples showed that the overall reproducibility of the method, measured by the coefficient of variation, ranged from 8.5 to 9.2% for nebicapone, 6.1 to 13.7% for nebicapone-glucuronide, and from 5.8 to 8.6% for 3-*O*-methyl-nebicapone. Also, the overall inaccuracy ranged from −4.9 to −0.8% for nebicapone, −4.7 to −2.3% for nebicapone-glucuronide, and from 0.1 to 7.0% for 3-*O*-methyl-nebicapone. The lower limit of quantification (LLOQ) of nebicapone, nebicapone-glucuronide and 3-*O*-methyl-nebicapone was 1044 ng/mL, 2609 ng/mL and 261 ng/mL, respectively. In previous studies[1,2], the LLOQ for nebicapone and 3-*O*-methyl-nebicapone was 50 ng/mL and 20 ng/mL, respectively. In the current study, the LLOQ was higher to allow the analysis of nebicapone-glucuronide in the same assay run.

Sample preparation for S-COMT assay

The same blood samples taken for the pharmacodynamic assessments were used for the preparation of washed erythrocytes for the assay of S-COMT activity. After centrifugation and removal of the resulting plasma, the upper most cell layer was removed and the tubes placed in ice, and a volume of cold 0.9% sodium chloride solution twice that of the cells was added. The cells were mixed, centrifuged and washed using this procedure three times. Centrifugation was undertaken at 4°C and at approximately 1500 *g* for 10 min. Two accurately pipetted aliquots of washed cells (250 µL) were retained (Series A and B) and each aliquot was stored in a 2 mL tube at −70°C until required for analysis. Likewise, blank erythrocytes for blank matrix preparation were obtained from normal volunteers and processed, transferred and stored using the same methods.

S-COMT activity was assayed under GLP conditions by the Laboratory of Pharmacological Research of Bial using a validated method consisting of HPLC with electrochemical detection (ED) and spectrophotometer analysis for quantification of the metanephrine (MN) and haemoglobin (Hb) content, respectively. S-COMT activity assay was based on catabolism of adrenaline to MN by erythrocyte S-COMT and erythrocyte Hb content. S-COMT activity was expressed as the amount of MN formed per mg of Hb per hour (pmol/mg Hb/h). After thawing on ice, erythrocyte specimens were haemolysed at a water to cell ratio of 3:1 v./v. to perform a crude extraction of S-COMT. After centrifugation to pellet the particulate debris, the resulting supernatant was used for S-COMT activity analysis and Hb protein determination. S-COMT activity was determined by introducing the erythrocyte supernatant into a reaction matrix containing the COMT substrate adrenaline, which is catabolised to MN. After termination of the reaction by addition of 0.4 M perchloric acid (PCA)

and centrifugation to pellet precipitated proteins, the amount of MN produced over the fixed reaction time of 60 min at 37°C was measured by HPLC-ED analysis (performed on a Gilson modular system) and interpolation with a MN standard curve to give the amount of MN formed (ng/mL). Hb determination was based on the oxidation of Hb and its derivatives to methaemoglobin in the presence of alkaline potassium ferricyanide and the subsequent reaction of methaemoglobin with potassium cyanide to form cyanmethaemoglobin, which has a maximum absorption at 540 nm. This reaction was achieved by addition of erythrocyte lysate supernatant to Drabkin's reagent under controlled conditions and quantification was performed by spectrophotometric analysis (performed on a Molecular Devices SpectraMAX Plus System) at 540 nm and interpolation with cyanmethaemoglobin standard curve to give Hb concentration (mg/mL) of the specimen (Sigma Diagnostics Total Haemoglobin assay kit, Sigma Procedure number 525).

For HPLC analysis, a MN calibration curve and QC samples were included in each HPLC-ED analytical run. The MN calibration curve consisted of at least three concentrations in triplicate within the linear range of the assay, prepared by spiking blank matrix with a known concentration of MN. For the Hb assay, the standard curve of Hb concentration (over the calibration range of 3–18 g/dL) consisted of at least four concentrations of cyanmethaemoglobin reference standard equivalent. Hb AC samples made up from cyanmethaemoglobin standard were included in each analysis. The QC samples were used to monitor the performance of the method for S-COMT activity determination. The analytical runs were accepted when not more than two QC specimens, and not two at the same level, deviate by more than 15% from the theoretical concentration. The overall reproducibility of the method of MN detection, measured by the coefficient of variation, ranged from 0.7 to 2.9% and the overall inaccuracy ranged from −9.3 to 8.2.

Pharmacokinetic and pharmacodynamic analysis

For each subject, the following pharmacokinetic parameters for nebicapone, nebicapone-glucuronide and 3-*O*-methyl-nebicapone were derived from the individual plasma concentration–time profiles using a non-compartmental approach: C_{max}; t_{max}; area under the plasma concentration–time curve from time 0 to last observed concentration at time t (AUC_{0-t}), calculated by the linear trapezoidal rule; AUC from time 0 to 4 h post-dose (AUC_{0-4}); AUC from time 0 to infinity ($AUC_{0-\infty}$); and apparent terminal elimination half-life ($t_{½}$), calculated from $ln\ 2/\lambda_z$, where λ_z is the apparent terminal elimination rate constant calculated by log-linear regression of the terminal segment of the concentration–time curve. Actual sampling times were used for the pharmacokinetic analysis. Special consideration was given to the estimation of λ_z and corresponding $t_{½}$ values. Values of λ_z were calculated from a minimum of three data points. Where an AUC was extrapolated to infinity, the percentage of the extrapolated area to the total area was assessed and if greater than 20% the AUC value was flagged as unreliable. Plasma concentrations below the LLOQ of the assay were taken as zero for all calculations. All calculations were made using raw data.

The following mean pharmacodynamic parameters 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) over the dosing interval ($AUEC_{0-4}$) following the first and last doses, and AUEC over 48 h ($AUEC_{0-48}$) following the last dose. The result obtained before the first dose was taken as reference (baseline) value (E_0). Maximum percent inhibition of S-COMT activity was calculated according to the equation $[(E_0 - E_{max})/E_0] \times 100$.

The pharmacokinetic and pharmacodynamic parameters were calculated using WinNonlin version 4.1 (Pharsight Co, Mountain View, CA, USA). The statistical computations were performed using SAS (release 8.2, SAS Institute Inc, Cary, NC, USA).

Results

Six young healthy Caucasian subjects (three males and three females) participated in each group. Their mean ± SD (range) age and BMI were, respectively: 23.7 ± 3.1 (21–28) years and 22.5 ± 2.8 (18.9–26.1) kg/m^2 in the nebicapone 100 mg group; 22.2 ± 0.4 (22–23) years and 22.2 ± 3.2 (18.6–27.1) kg/m^2 in the nebicapone 200 mg group; and 24.3 ± 5.4 (18–32) and 24.4 ± 2.5 (20.6–27.5) kg/m^2 in the placebo group.

Pharmacokinetic results

Figures 1–3 display the mean plasma concentration–time profiles of nebicapone, nebicapone-glucuronide and 3-*O*-methyl-nebicapone, respectively.

Mean pharmacokinetic parameters of nebicapone and its metabolites following the first and last doses of nebicapone 100 mg and 200 mg are summarised in Table 1. Nebicapone appeared to be extensively metabolised to its glucuronide metabolite, being the nebicapone-glucuronide the major early circulating drug entity. However, due to its long half-life and accumulation, 3-*O*-methyl-nebicapone was the major late metabolite in circulation. For a nebicapone dose increase in the ratio 1.0:2.0, nebicapone AUC_{0-4} and C_{max} values increased respectively

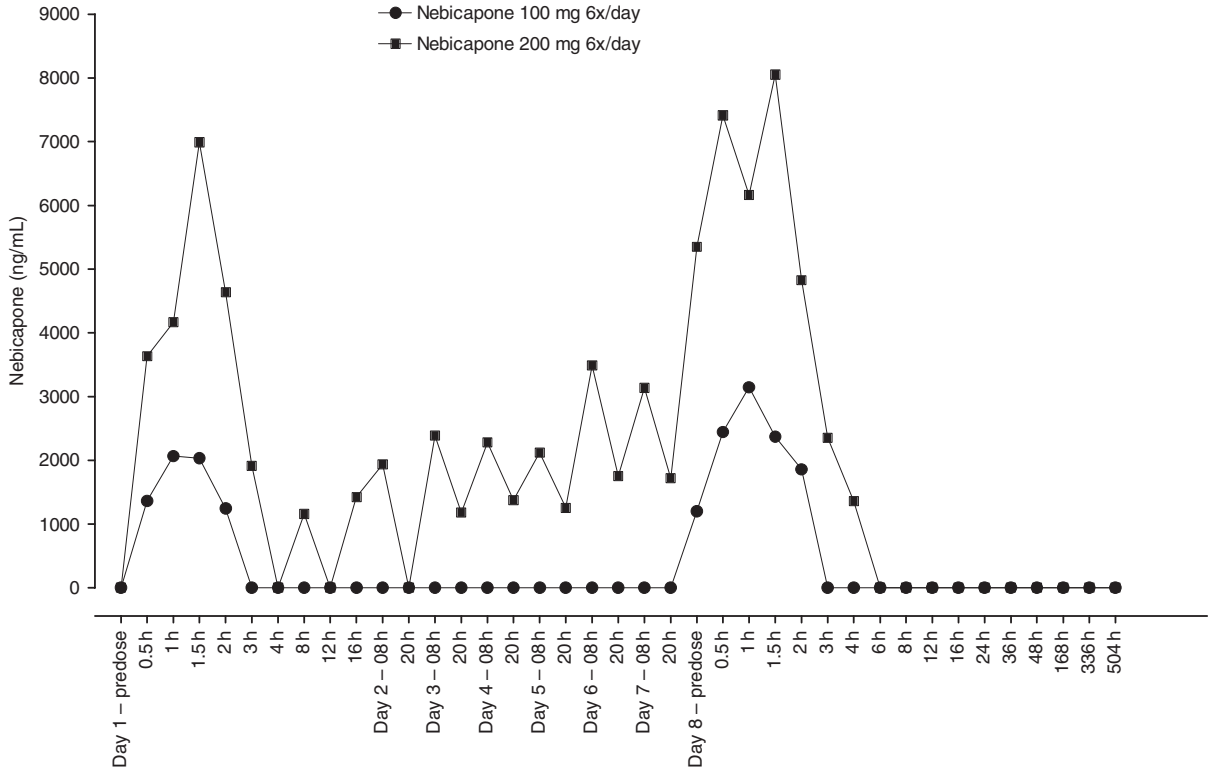

Figure 1. Mean plasma concentration–time profile of nebicapone during a 7-day treatment with nebicapone 100 mg and 200 mg administered at 4-h intervals ($n = 6$ per dose). From the 4-h (day 1) time-point until the pre-final dose (day 8) time-point, blood samples were taken at 'trough' (pre-dose).

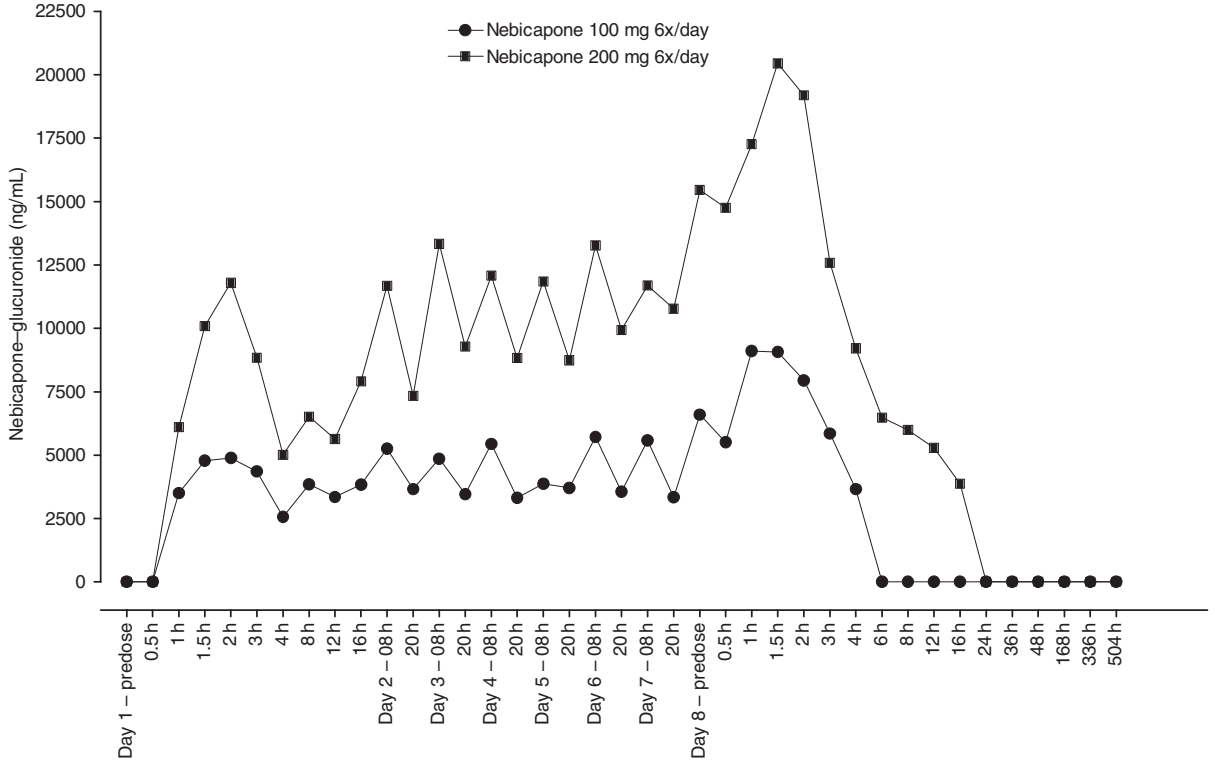

Figure 2. Mean plasma concentration–time profile of nebicapone-glucuronide during a 7-day treatment with nebicapone 100 mg and 200 mg administered at 4-h intervals ($n = 6$ per dose). From the 4 h (day 1) time-point until the pre-final dose (day 8) time-point, blood samples were taken at 'trough' (pre-dose).

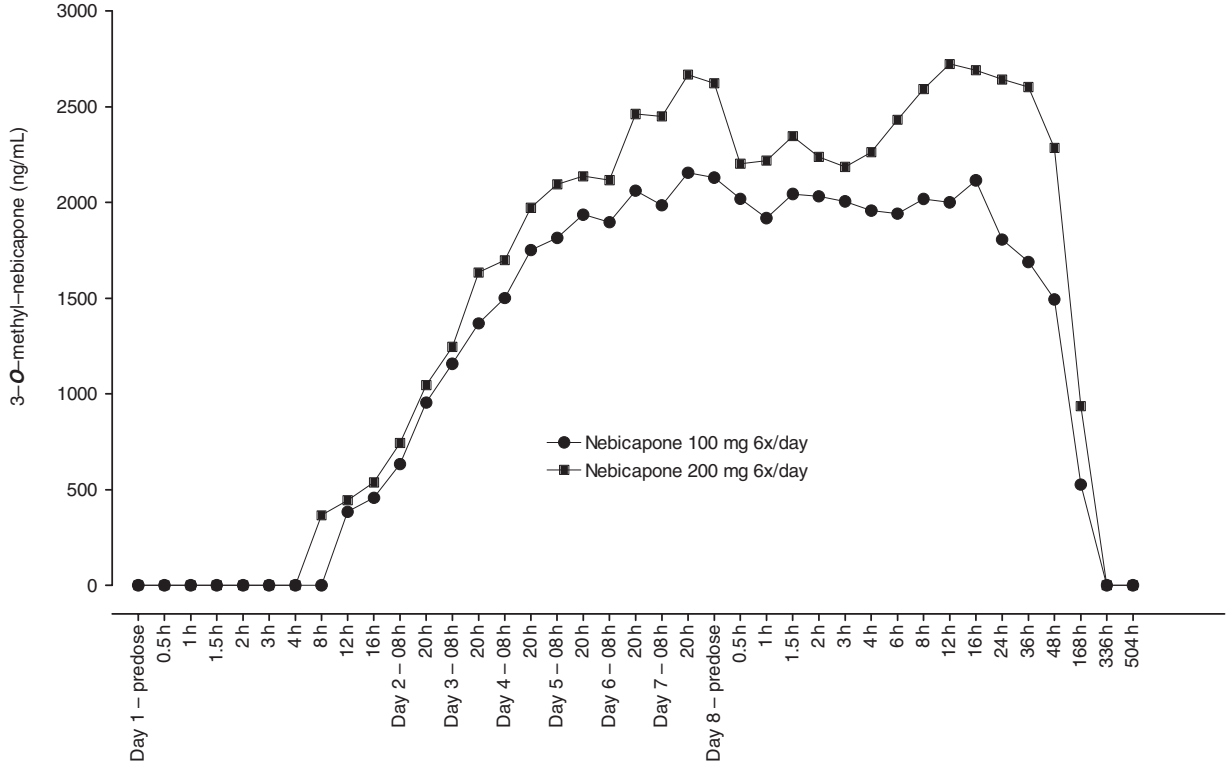

Figure 3. Mean plasma concentration–time profile of 3-*O*-methyl-nebicapone during a 7-day treatment with nebicapone 100 mg and 200 mg administered at 4-h intervals ($n = 6$ per dose). From the 4 h (day 1) time-point until the pre-final dose (day 8) time-point, blood samples were taken at 'trough' (pre-dose).

Table 1. Arithmetic mean ± SD pharmacokinetic parameters of nebicapone, nebicapone-glucuronide and 3-*O*-methyl-nebicapone following the first and last doses of a 7-day treatment with nebicapone 100 mg and 200 mg administered at 4-h intervals ($n = 6$ per dose).

Analyte	Nebicapone dose	Dosing time	C_{max} (ng/mL)	t_{max} (h)	AUC_{0-4} (ng.h/mL)	$AUC_{0-\infty}$ (ng.h/mL)	$t_{½}$ (h)
Nebicapone	100 mg	First dose	3837 ± 1839	1.3 (1.0–3.0)	4059 ± 1936	NC	NC
		Last dose	3349 ± 610	1.0 (0.5–2.0)	6041 ± 1750	8652 ± 2175	1.2 ± 0.2
	200 mg	First dose	8938 ± 2865	1.5 (0.5–1.5)	11 899 ± 4522	14 594 ± 4748	0.8 ± 0.2
		Last dose	10 915 ± 4859	1.3 (0.5–1.5)	18 721 ± 8266	30 632 ± 22 800	3.5 ± 3.9
Nebicapone-glucuronide	100 mg	First dose	7195 ± 2137	1.8 (1.0–4.0)	11 904 ± 5935	22 461 ± 2840	1.3 ± 0.1
		Last dose	9779 ± 2005	1.0 (0.0–1.5)	27 125 ± 4498	40 595 ± 8061	2.6 ± 1.4
	200 mg	First dose	12 643 ± 2851	1.8 (1.0–2.0)	29 076 ± 9335	44 836 ± 23 465	1.9 ± 1.1
		Last dose	22 626 ± 8028	1.3 (0.0–2.0)	61 657 ± 22 471	20 0781 ± 67 611	12.7 ± 6.4
3-*O*-methyl-nebicapone	100 mg	First dose	NC	NC	NC	NC	NC
		Last dose	2216 ± 288	1.5 (0.0–4.0)	8031 ± 936	299 543 ± 52 433	93.3 ± 11.2
	200 mg	First dose	314 ± 37	4.0 (4.0–4.0)	351 ± 186	NC	NC
		Last dose	2629 ± 302	0.0 (0.0–3.0)	9035 ± 870	459 825 ± 73 576	97.6 ± 21.0

t_{max} are median values with range in parenthesis; NC, not calculated because the parameter could not be reliably estimated (no sufficient data points available during the mono-exponential phase).

1.0:2.9 and 1.0:2.3 following the first dose and 1.0:3.1 and 1.0:3.3 following the last dose.

Pre-dose plasma nebicapone concentrations following nebicapone 100 mg were below the LLOQ, but pre-dose plasma concentrations following nebicapone 200 mg before the 8:00 AM and 8:00 PM doses across days 1 to 8 showed a circadian variation in nebicapone and nebicapone-glucuronide plasma concentrations (Table 2). Pre-dose plasma concentrations of nebicapone and nebicapone-glucuronide across days 2–8 suggested that steady state was reached at least by day 2. The data do not assure that the steady state for 3-*O*-methyl-nebicapone was reached during the study.

Pharmacodynamic results

Mean COMT inhibition profiles and pharmacodynamic parameters for COMT inhibition following administration

Table 2. Mean ± SD plasma concentrations of nebicapone, nebicapone-glucuronide and 3-*O*-methyl-nebicapone before (pre-dose) the 8:00 AM and 8:00 PM doses of a 7-day treatment with nebicapone 100 mg and 200 mg administered at 4-h intervals ($n = 6$ per dose).

Nebicapone dose	Day of treatment	Dosing time	Nebicapone (ng/mL)	Nebicapone-glucuronide (ng/mL)	3-*O*-methyl-nebicapone (ng/mL)
100 mg	1	8:00 AM	BLQ	BLQ	BLQ
		8:00 PM	BLQ	3412 ± 1816	BLQ
	2	8:00 AM	BLQ	5254 ± 1696	634 ± 104
		8:00 PM	BLQ	3254 ± 1621	955 ± 144
	3	8:00 AM	BLQ	4853 ± 1441	1158 ± 185
		8:00 PM	BLQ	3102 ± 1517	1368 ± 193
	4	8:00 AM	BLQ	5441 ± 1448	1501 ± 210
		8:00 PM	BLQ	2913 ± 1443	1751 ± 219
	5	8:00 AM	BLQ	3868 ± 682	1815 ± 212
		8:00 PM	BLQ	3322 ± 1784	1937 ± 256
	6	8:00 AM	BLQ	5718 ± 1403	1897 ± 274
		8:00 PM	BLQ	3554 ± 2197	2061 ± 221
	7	8:00 AM	BLQ	5584 ± 2543	1986 ± 213
		8:00 PM	BLQ	3339 ± 1693	2155 ± 207
	8	8:00 AM	BLQ	6595 ± 786	2130 ± 330
200 mg	1	8:00 AM	BLQ	BLQ	BLQ
		8:00 PM	1159 ± 946	6509 ± 2857	366 ± 80
	2	8:00 AM	1938 ± 1620	11 675 ± 5504	744 ± 99
		8:00 PM	BLQ	7339 ± 3167	1046 ± 164
	3	8:00 AM	2211 ± 1319	13 326 ± 6768	1246 ± 182
		8:00 PM	1179 ± 896	9273 ± 3895	1635 ± 275
	4	8:00 AM	2116 ± 1493	12 072 ± 4494	1698 ± 267
		8:00 PM	1218 ± 659	8830 ± 3032	1973 ± 207
	5	8:00 AM	2120 ± 1524	11 850 ± 5383	2094 ± 241
		8:00 PM	1255 ± 935	8741 ± 2771	2137 ± 296
	6	8:00 AM	3487 ± 3892	13 267 ± 6295	2116 ± 232
		8:00 PM	1751 ± 378	9932 ± 3012	2462 ± 276
	7	8:00 AM	3136 ± 1787	11 680 ± 4255	2450 ± 182
		8:00 PM	1717 ± 312	10 773 ± 4062	2667 ± 293
	8	8:00 AM	5349 ± 4264	15 450 ± 4075	2623 ± 282

BLQ, below the lower limit of quantification.

of placebo, nebicapone 100 mg and 200 mg are summarised in Figure 4 and Table 3. Both nebicapone 100 mg and 200 mg doses caused a marked inhibition of S-COMT (Figure 5). Peak S-COMT activity inhibition was 67% and 88% following the first dose of nebicapone 100 mg and 200 mg, respectively, and occurred between 1.3 and 1.9 h post-dose. Following repeated administration, peak inhibition of S-COMT activity was 70% and 87%, respectively, and occurred at approximately 0.9–1.3 h post-dose. The circadian variation found in the pre-dose plasma concentrations of nebicapone (see Figure 1) and nebicapone-glucuronide (see Figure 2) was also apparent in S-COMT activity. The S-COMT activity was lower (less MN formed) at the 8:00 AM pre-dose assay, a time at which 'trough' nebicapone levels were maximal (Figure 6). No circadian change in S-COMT was observed in the placebo group showing that the variation in S-COMT activity found in the nebicapone groups was due to the circadian change in nebicapone plasma levels.

Tolerability/safety results

Treatment-emergent adverse events (AEs) are listed in Table 4. Four subjects (66.7%) in the nebicapone 100 mg and placebo groups and six subjects (100%) in the nebicapone 200 mg group reported at least one AE. All AEs were of mild or moderate intensity. At discharge, alanine transaminase (ALT) values rose to above three times the upper limit of the normal range (ULN) in one male subject of the nebicapone 100 mg group (190 IU/L, normal range: 5–40 IU/L) and one female subject of the nebicapone 200 mg group (188 IU/L, normal range: 5–31 IU/L) and the abnormality was considered to be clinically significant. In both cases, the ALT level at admission was above the ULN (88 and 60 IU/L, respectively) but the clinical investigator considered the abnormality as not clinically relevant. Bilirubin values remained within the normal range. There were no clinically significant abnormalities in vital signs, or in physical examination or ECG parameters.

Discussion

The present study aimed to investigate the tolerability, safety, pharmacokinetics and pharmacodynamics of nebicapone during a 7-day treatment period in which nebicapone was administered at 4-h intervals (6 times/day). Available COMT inhibitors are tolcapone and entacapone. Tolcapone is used twice- or thrice-daily; entacapone is used in co-administration with each dose

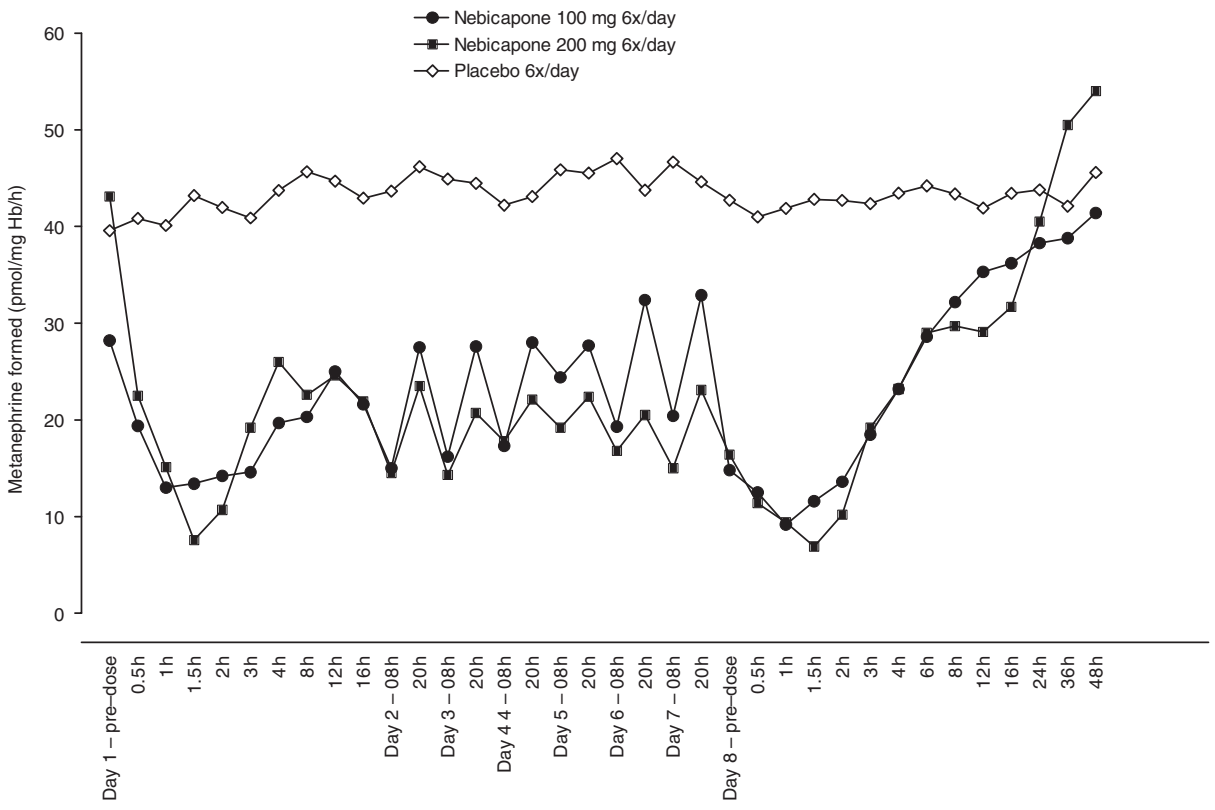

Figure 4. Mean S-COMT activity (expressed as metanephrine formed, pmol/mg Hb/h) profile from baseline (pre-dose) during a 7-day treatment with nebicapone 100 mg, nebicapone 200 mg and placebo administered at 4-h intervals ($n = 6$ per dose). From the 4 h (day 1) time-point until the pre-final dose (day 8) time-point, blood samples were taken at 'trough' (pre-dose).

Table 3. Mean ± SD pharmacodynamic parameters for inhibition of S-COMT following the last dose of a 7-day treatment with nebicapone 100 mg and 200 mg administered at 4-h intervals ($n = 6$ per dose).

Nebicapone dose	Dosing time	E_0 (pmol/mg Hb/h)	E_{max} (pmol/mg Hb/h)	t_{Emax} (h)	$[(E_0 - E_{max})/E_0] \times 100$(%)	$AUEC_{0-4}$ (pmol/mg Hb/h.h)
100 mg	First dose	28.2 ± 12.2	7.18 ± 1.17	1.9 ± 1.3	67.1 ± 24.7	61.1 ± 21.3
	Last dose	–	8.22 ± 4.56	1.3 ± 0.9	70.1 ± 9.2	63.9 ± 20.6
200 mg	First dose	43.2 ± 20.3	5.80 ± 4.64	1.3 ± 0.5	87.7 ± 4.7	73.6 ± 42.1
	Last dose	–	6.00 ± 5.03	0.9 ± 0.5	87.2 ± 5.8	63.1 ± 34.7

of levodopa/carbidopa or levodopa/benserazide, which can occur up to 8 times per day, but not usually during the sleep time. In the current study, nebicapone was administered even during the night time, at strict time intervals, in order to facilitate the evaluation of its chronopharmacology.

Nebicapone appeared to be extensively metabolised to its glucuronidated and 3-O-methylated metabolites, being the glucuronide the major early circulating drug entity and the 3-O-methylated the major late metabolite. The mean nebicapone and nebicapone-glucuronide plasma concentrations before the 8 PM dose were approximately 60% those before the 8 AM dose, showing a clear circadian variation of nebicapone pharmacokinetics (chronopharmacokinetics).

Circadian variations in gastric acid secretion and pH, motility, gastric emptying time, gastrointestinal blood flow, drug protein binding, liver enzyme activity and/or hepatic blood flow, glomerular filtration, renal blood flow, urinary pH and tubular reabsorption may be responsible for circadian changes in the absorption, distribution, metabolism or elimination of drugs[11]. In the current results, it is interesting to note that the highest and lowest levels of parent drug (nebicapone) and its glucuronide occurred at the same time of day. In consequence, it can be concluded that the decrease in nebicapone levels found in the evening was not due to over-formation of its glucuronide conjugate, because levels of this metabolite were also lower at evening. Since the nebicapone level decrease in the evening cannot also be attributed to

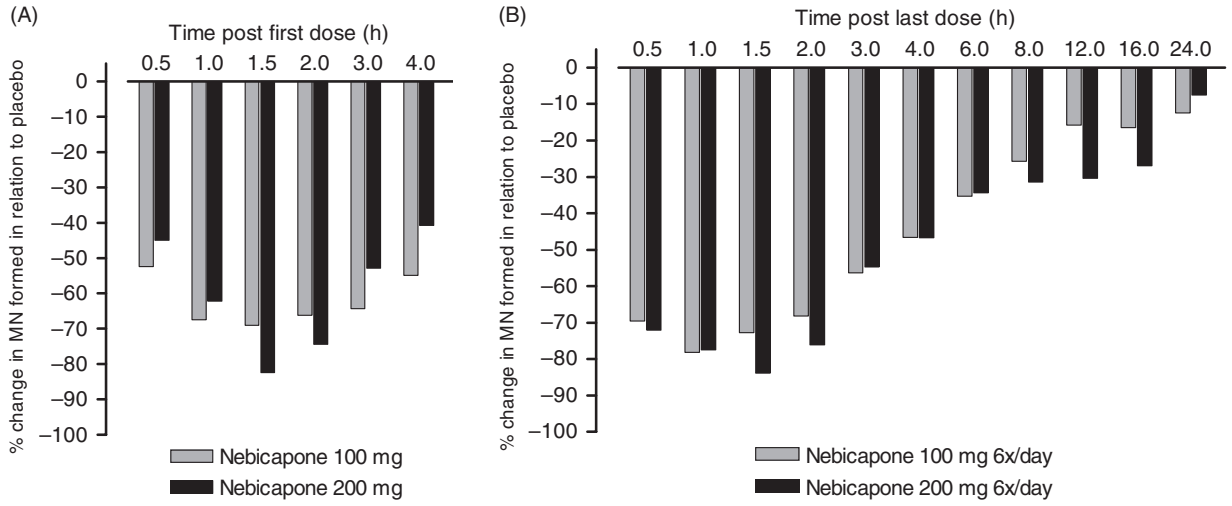

Figure 5. Relative change of the mean metanephrine (MN) formed in relation to placebo following the first dose (A) and the last dose (B) of a 7-day treatment with nebicapone 100 mg, nebicapone 200 mg and placebo administered at 4-h intervals ($n = 6$ per dose).

over-formation of 3-O-methyl-nebicapone, one can hypothesise that the circadian variation of nebicapone levels may be due to differences at the absorption level. Since a previous study[2] showed that nebicapone pharmacokinetics are not affected by the presence of food and in the current study nebicapone was administered in fasting and under strict control of diet and other extrinsic factors, variation in absorption is most likely due to intrinsic factors that suffer circadian variation and that may affect the absorption of nebicapone. It is not known whether other COMT inhibitors also present chronopharmacokinetics.

No variation was apparent for 3-O-methylated-nebicapone, but this metabolite has a long elimination half-life and accumulates and, therefore, an eventual circadian variation in its formation hardly would be apparent in the plasma concentration–time profiles.

S-COMT activity showed no circadian variation in the placebo group. Therefore, the circadian variation found in S-COMT activity in the nebicapone-treated subjects is likely due to the circadian changes in plasma concentrations of nebicapone, which is consistent with a pre-dose S-COMT activity lower at the time at which nebicapone levels were maximal.

Drug chronopharmacology may be clinically relevant as it may have implications for drug prescription by modulating the distribution of the total daily dose along the 24-h scale[11]. The clinical importance of the circadian variation in the nebicapone metabolism and activity has not yet been studied in Parkinson's disease patients but deserves further evaluation. In case the circadian variation will have a relevant impact on the therapeutic outcomes, higher doses of nebicapone may be necessary at the end of the day in comparison with the morning doses.

One of the limitations of this study is the fact that all study participants were Caucasian and young. It is unknown whether the results of this study can be generalised to non-Caucasian and elderly subjects.

Clinically relevant elevations in AST were observed in one subject of each nebicapone group. However, in both cases the ALT levels were found to be above the upper limit of the normal range at admission to the study and bilirubin values remained within the normal range. Several studies with nebicapone in healthy subjects[1–7] and in PD patients[12], in which more than 180 subjects were exposed to different dosage regimens of nebicapone, did not raise any liver safety concerns. Overall, the previous data do not suggest a potential hepatotoxic effect of nebicapone. However, liver enzyme increase was reported by 1–3% of patients in phase II/III trials[13–15] with tolcapone and there are several cases of acute hepatotoxicity that were attributed to this COMT inhibitor[16]. Therefore, the risk of increasing liver transaminases by nebicapone and its clinical relevance deserves further evaluation.

Conclusion

Nebicapone showed chronopharmacology in young Caucasian healthy subjects. The clinical importance of the circadian variation in the nebicapone metabolism and activity in Parkinson's disease patients has not yet been studied and deserves evaluation as it may have implications for drug prescription by modulating the distribution of the total daily dose along the 24-h scale. Both nebicapone treatment regimens tested were subjectively well-tolerated; however, two cases of significant increase of AST were reported.

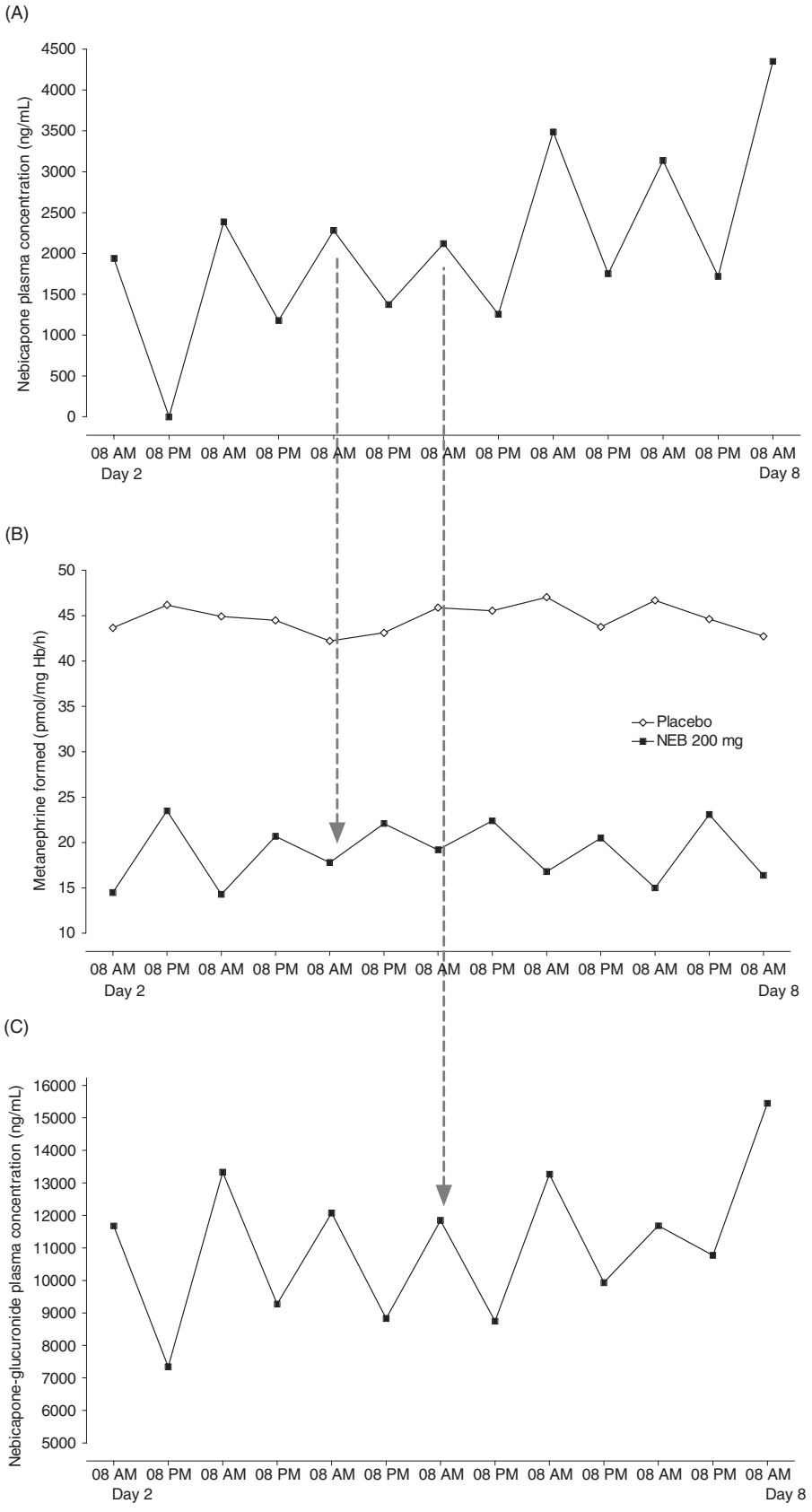

Figure 6. Nebicapone (A) and nebicapone-glucuronide (C) plasma concentrations and S-COMT activity (B) before (pre-dose) the 8:00 AM and 8 PM doses of a 7-day treatment with nebicapone 200 mg administered at 4-h intervals.

Table 4. Number (percentage) of subjects reporting adverse events considered possibly related to treatment.

Adverse events (lowest level term, MedDRA dictionary)	Nebicapone 100 mg 6×/day ($n=6$)	Nebicapone 200 mg 6×/day ($n=6$)	Placebo ($n=6$)
Abdominal cramps	0	1 (16.7%)	0
ALT increased	1 (16.7%)	1 (16.7%)	0
Chest pain (non-cardiac)	1 (16.7%)	1 (16.7%)	0
Dry throat	0	0	1 (16.7%)
Epigastric pain not food-related	0	1 (16.7%)	0
Groggy on awakening	0	1 (16.7%)	0
Headache	1 (16.7%)	2 (33.3%)	1 (16.7%)
Heartburn	1 (16.7%)	4 (66.7%)	2 (33.3%)
Low back pain (without radiation)	1 (16.7%)	0	0
Lower abdominal pain	0	1 (16.7%)	0
Macular rash and local anaesthesia	0	1 (16.7%)	0
Myalgia of lower extremities	1 (16.7%)	0	0
Nausea	1 (16.7%)	1 (16.7%)	0
Nausea and abdominal cramps	0	1 (16.7%)	0
Nausea and vomiting	1 (16.7%)	0	0
Nausea and sweating	0	0	1 (16.7%)
Otalgia	0	1 (16.7%)	0
Postural hypotension	0	0	0
Rhinorrhoea	0	1 (16.7%)	0
Upper abdominal pain	0	1 (16.7%)	0
Visual disturbances subjective	1 (16.7%)	1 (16.7%)	
Visual impairment transient	1 (16.7%)	0	0
Total	4 (66.7%)	6 (100.0%)	4 (66.7%)

Some subjects reported more than one AE.

Transparency

Declaration of funding

This trial was funded by Bial (Portela & C[a], SA, S. Mamede do Coronado, Portugal). Bial was responsible for both the design and the conduct of the study. Bial funded the statistical and pharmacokinetic/pharmacodynamic analysis of this manuscript. The first author was responsible for the first and final versions of the manuscript. All authors had full access to the data and substantially contributed to the interpretation of the data and the writing of the manuscript. L.A. and P. Soares-da-Silva were responsible for the study design and study supervision. M. Vaz-da-Silva and J.M. were responsible for the clinical conduct. A.I.L., L.T., C. Fernandes-Lopes, B.I. and L.W. were responsible for the determination of drug plasma concentrations and S-COMT assays. A.F. conducted *the pharmacokinetic/pharmacodynamic analysis*.

Declaration of financial/other relationships

All authors but A.F. *are or were employees of Bial (the sponsor of the study) at the time of the study. A.F. is an employee of 4Health, an independent contract research organisation contracted by the sponsor to conduct the pharmacokinetic/pharmacodynamic analysis*.

Peer reviewers may receive honoraria for their review work at CMRO. The peer reviewers have disclosed no relevant financial relationships.

Acknowledgements

This study was sponsored by Bial, Portugal. Data management was carried out by the independent contract research organisation, Eurotrials – scientific consultants (sponsored by Bial). The pharmacokinetic/pharmacodynamic analysis was carried out by the independent contract research organisation, 4Health (sponsored by Bial).

The authors *thank the healthy subjects who participated in the study and the clinical and laboratory staff of Bial's Human Pharmacology Unit*.

References

1. Almeida L, Soares-da-Silva P. Pharmacokinetics and pharmacodynamics of BIA 3-202, a novel COMT inhibitor, during first administration to humans. Drugs R D 2003;4:207-17
2. Almeida L, Soares-da-Silva P. Pharmacokinetic and pharmacodynamic profiles of BIA 3-202, a novel catechol-O-methyltransferase (COMT) inhibitor, during multiple-dose administration to healthy subjects. J Clin Pharmacol 2003;43:1350-60
3. Silveira P, Vaz-da-Silva M, Almeida L, et al. Pharmacokinetic-pharmacodynamic interaction between BIA 3-202, a novel COMT inhibitor, and levodopa/benserazide. Eur J Clin Pharmacol 2003;59:603-9
4. Almeida L, Vaz-da-Silva M, Silveira P, et al. Pharmacokinetic-pharmacodynamic interaction between BIA 3-202, a novel COMT inhibitor, and levodopa/carbidopa. Clin Neuropharmacol 2004;27:17-24
5. Vaz-da-Silva M, Loureiro AI, Nunes T, et al. Pharmacokinetic-pharmacodynamic interaction between nebicapone, a novel catechol-O-methyltransferase inhibitor, and controlled-release levodopa/carbidopa 200 mg/50 mg: randomized, double-blind, placebo-controlled, crossover study in healthy subjects. Drugs R D 2008;9:435-46
6. Nunes T, Machado R, Rocha JF, et al. Pharmacokinetic-pharmacodynamic interaction between nebicapone, a novel COMT inhibitor, and controlled release levodopa/benserazide: a single-centre, phase I, double-blind, randomized, placebo-controlled, four-way crossover study in healthy subjects. Clin Ther 2009;31:2258-71

7. Almeida L, Falcao A, Vaz-da-Silva M, et al. Effect of nebicapone on the pharmacokinetics and pharmacodynamics of warfarin in healthy subjects. Eur J Clin Pharmacol 2008;64:961-6
8. Ferreira J, Almeida L, Cunha L, et al. Effects of nebicapone on levodopa pharmacokinetics, catechol-O-methyltransferase (COMT) activity and motor fluctuations in patients with Parkinson's disease. Clin Neuropharmacol 2008;31:2-18
9. Loureiro AI, Bonifacio MJ, Fernandes-Lopes C, et al. Human metabolism of nebicapone (BIA 3-202), a novel catechol-O-methyltransferase inhibitor: characterization of in vitro glucuronidation. Drug Metab Dispos 2006;34:1856-62
10. Palma PN, Bonifacio MJ, Almeida L, et al. Restoring dopamine levels. In: Smith HJ, Simons C, Sewell RDE, eds. Protein Misfolding in Neurodegenerative Diseases. Boca Raton, FL, USA: CRC Press, 2008:415-45
11. Bruguerolle B. Chronopharmacokinetics current status. Clin Pharmacokinet 1998;35:83-94
12. Ferreira JJ, Almeida L, Cunha L, et al. Effects of nebicapone on levodopa pharmacokinetics, catechol-O-methyltransferase activity, and motor fluctuations in patients with Parkinson disease. Clin Neuropharmacol 2008;31:2-18
13. Baas H, Beiske AG, Ghika J, et al. Catechol-O-methyltransferase inhibition with tolcapone reduces the "wearing off" phenomenon and levodopa requirements in fluctuating parkinsonian patients. J Neurol Neurosurg Psychiatry 1997;63:421-8
14. Rajput AH, Martin W, Saint-Hilaire MH, et al. Tolcapone improves motor function in parkinsonian patients with the "wearing-off" phenomenon: a double-blind, placebo-controlled, multicenter trial. Neurology 1997; 49:1066-71
15. Waters CH, Kurth M, Bailey P, et al. Tolcapone in stable Parkinson's disease: efficacy and safety of long-term treatment. The Tolcapone Stable Study Group. Neurology 1997;49:665-71
16. Olanow CW. Tolcapone and hepatotoxic effects. Tasmar Advisory Panel. Arch Neurol 2000;57:263-7