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Determination of cabergoline and L-dopa in human plasma using liquid chromatography–tandem mass spectrometry

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Abstract

We determined cabergoline and L-dopa in human plasma using liquid chromatography–mass spectrometry with tandem mass spectrometry (LC–MS–MS). The deproteinized plasma samples with organic solvent or acid were analyzed directly by reversed-phase liquid chromatography. Using multiple reaction monitoring (MRM, product ions m/z 381 of m/z 452 for cabergoline and m/z 152 of m/z 198 for L-dopa) on LC–MS–MS with electrospray ionization (ESI), cabergoline and L-dopa in human plasma were determined. Calibration curves of the method showed a good linearity in the range 5–250 pg/ml for cabergoline and 1–200 ng/ml for L-dopa, respectively. The limit of determination was estimated to be approximately 2 pg/ml for cabergoline and approximately 0.1 ng/ml for L-dopa, respectively. The method was applied to the analysis of cabergoline and L-dopa in plasma samples from patients treated with these drugs. The precision of analysis showed coefficients of variation ranging from 3.8% to 10.5% at cabergoline concentration of 13.8–26.2 pg/ml and from 2.9% to 8.9% at an L-dopa concentration of 302.5–522.1 ng/ml in patient plasma. As a result, the procedure proved to be very suitable for routine analysis.

Keywords: L-Dopa; Cabergoline

1. Introduction

Cabergoline, *N*-[3-(dimethylamino)propyl]-*N*-(ethylamino)carbonyl-6-(2-propenyl)-ergoline-8β-carboxamide (Fig. 1) is an ergot alkaloid derivative with dopamine agonist activity [1]. It has been shown to induce long-lasting inhibition of prolactin secretion in rats [2] and in hyperprolactinemia patients [3]. Moreover, it has been reported that cabergoline improves Parkinson symptoms in MPTP-treated monkeys [4] and is effective in the treatment of Parkinson's disease [5,6].

An oral dose of cabergoline lower than 1 mg/day is usually used for the treatment of Parkinson's disease, and it has been reported that the plasma levels of cabergoline in healthy volunteers who took a single oral dose of 0.6 mg is in the range 80–800 pg/ml using the radioimmunoassay method [7]. The very low dosages in humans has required the development of a more sensitive and selective method for measuring plasma levels of cabergoline. Al-

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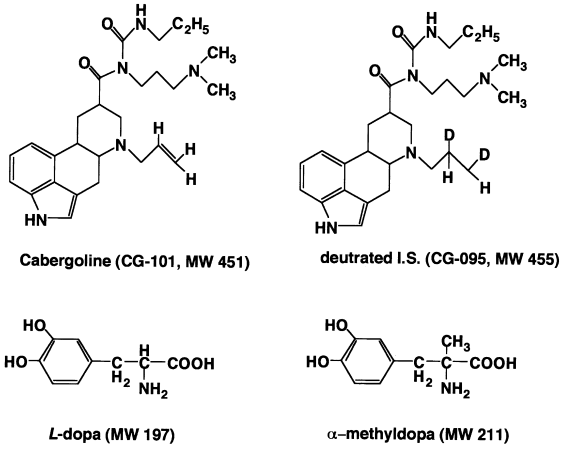

Fig. 1. Chemical structures of cabergoline, deuterated cabergoline (I.S.), L-dopa and α-methyldopa (I.S.).

though an RIA method was developed to quantify cabergoline in plasma samples, it is thought that its method cannot give a satisfactory result because its major metabolite, *N*-demethyl metabolite, cross-reacts with the antibody. For quantitative analysis, a liquid chromatography–mass spectrometry (LC–MS) method has recently become a powerful technique. In addition, the application of tandem mass spectrometry (MS–MS) has improved both the sensitivity and selectivity of the determination. A large number of studies have been performed using liquid chromatography combined with tandem mass spectrometry (LC–MS–MS) for the determination of drugs in biological fluids [8–13]. This method has recently been used for the determination of cabergoline in human plasma [14]. In the present study, we also developed the modified method to determine plasma levels of cabergoline using LC–MS–MS.

Combination treatment of L-dopa with cabergoline has been used for the treatment of Parkinson's disease. In this case, it is important that the plasma concentration of L-dopa is determined in the patients administered with cabergoline. Although many methods have been developed for the determination of L-dopa in plasma, reversed-phase high performance liquid chromatography (HPLC) with electrochemical detection (ECD) [15–18] is currently used. In the present study, the analytical procedure using LC–MS–MS was applied to the analysis of L-dopa in human plasma samples and developed.

2. Experimental

2.1. Chemicals and reagents

Cabergoline and deuterated internal standard (CG-095, I.S.) (Fig. 1) were provided by Kissei Pharmaceutical Co. (Matsumoto, Japan). L-Dopa and α-methyldopa (I.S.) were obtained from Sigma Chemical Co. (St. Louis, USA). Acetonitrile (HPLC grade), methanol (HPLC grade), high purity ammonium formate and formic acid were obtained from NACALAI Tesque Inc. (Kyoto, Japan). All other chemicals and reagents were of analytical grade from commercial sources.

2.2. Sample collection

Four schizophrenic patients (41–63 years old) volunteered to take part in this study; informed consent was obtained from each subject. Blood samples were taken 1 h after oral administration of the drug (cabergoline 0.25–1.0 mg or L-dopa 200 mg) and placed in heparinized tubes. The plasma was separated by centrifugation and stored at −40 °C until analysis.

Plasma samples from healthy volunteers were also used as control for development of extraction procedure for cabergoline and L-dopa.

2.3. Sample preparation for LC–MS

For determination of cabergoline, plasma samples (100 μl) were mixed with 50 μl of acetonitrile, 20 m*M* ammonium formate solution (90:10, v/v) and 50 μl of I.S. solution (CG-095, 1 ng/ml in acetonitrile) in the microcentrifuge tubes. It was then centrifuged at 10 000 *g* for 10 min and the supernatant was filtered with a 0.45-μm filter. The filtrate was transferred to the autosampler vial insert, and 30 μl was injected into the LC–MS–MS system.

For determination of L-dopa, plasma samples (50 μl) were added to 60 μl of 0.4 *M* perchloric acid and 40 μl of I.S. solution (α-methyldopa, 100 ng/ml in 0.4 *M* perchloric acid) in the microcentrifuge tubes. After shaking the tube for 1 min, it was centrifuged at 10 000 *g* for 10 min and the supernatant was filtered with a 0.45-μm filter. The filtrate was diluted with 100 μl of the HPLC mobile phase solution, and

was then transferred to the autosampler vial insert where 20 μl was injected into the LC–MS–MS system.

2.4. LC–MS–MS

LC–MS–MS was performed using a Quattro Ultima triple quadrupole tandem mass spectrometer (Micromass Inc., Manchester, UK) equipped with electrospray ionization (ESI). The HPLC system consisted of a Waters Alliance 2690 pump equipped with an autosampler (Waters Co., Milford, MA, USA) and the reversed-phase COSMOSIL 5C8-MS column (2 mm I.D.×150 mm, 5-μm particle diameter, NACALAI Tesque Inc., Kyoto, Japan) for cabergoline analysis or the reversed-phase COSMOSIL $5C_{18}$-MS column (2 mm I.D.×150 mm, 5-μm particle diameter, NACALAI Tesque Inc., Kyoto, Japan) for L-dopa analysis. The flow-rate of mobile phase for cabergoline analysis was set at 0.2 ml/min, with gradient elution starting at 20% acetonitrile–80% water (containing 20 m*M* ammonium formate) for 3 min, followed by a linear increase to 80% acetonitrile in 1 min, and held there for 10 min before rapidly returning to the initial conditions. For L-dopa analysis, a mobile phase consisting of 10% methanol–90% water (containing 05% formic acid) was delivered at a flow-rate of 0.2 ml/min. In both case, sample introduction to the mass spectrometer was stopped for 0–6 min for cabergoline and for 0–2 min for L-dopa using a switching valve after injection into the system. Ionization conditions for LC–MS–MS were as follows: for cabergoline, capillary voltage 2.7 kV, cone voltage 35 V, collision energy 18 eV, source temperature 80 °C, desolvation temperature 200 °C; for L-dopa, capillary voltage 3.2 kV, cone voltage 15 V, collision energy 14 eV, source temperature 80 °C, desolvation temperature 300 °C, and argon was used as collision gas.

3. Results and discussion

The ESI mass spectra of cabergoline and CG-095 (I.S.) are shown in Fig. 2. Cabergoline and CG-095 showed a protonated molecule ion $(M+H)^+$ of m/z 452 and 456, respectively. These $(M+H)^+$ ions were predominant on the ESI mass spectra. Investigation of the MS/MS behavior of cabergoline indicated that the product ion m/z 381 of cabergoline (m/z 452) was the most suitable ion for quantitative determination by multiple reaction monitoring (MRM) (Fig. 3). Moreover, the product ion m/z 385 was chosen as the most adequate ion of deuterated cabergoline (I.S.). Fig. 4a shows the MRM chromatograms of cabergoline (125 pg/ml) and I.S. standard solutions using the product ions with m/z 381 and 385, respectively. The MRM chromatograms of plasma obtained from patients who were treated with cabergoline are shown in Fig. 4b. It was a clean chromatogram free from interfering contaminants.

For the determination of cabergoline in human plasma, liquid–liquid extraction was first used as the

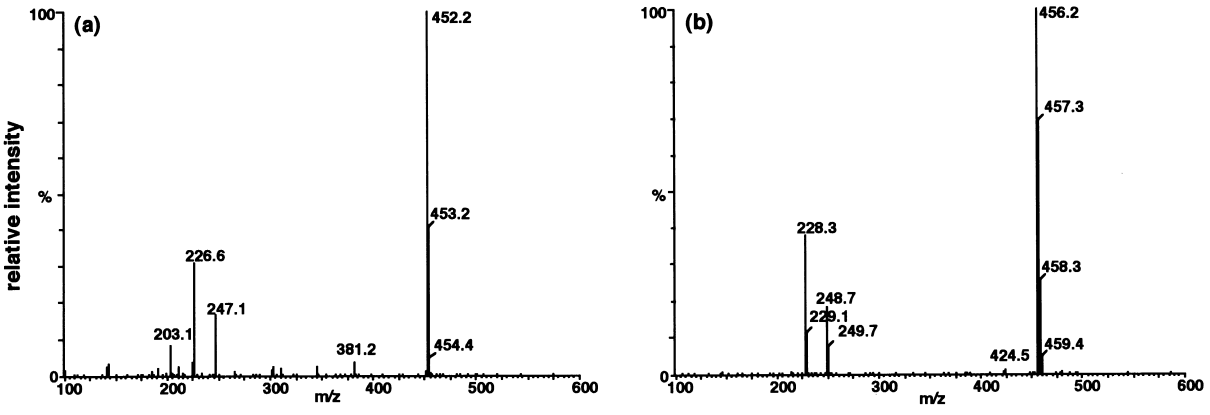

Fig. 2. LC–ESI mass spectra of cabergoline (a) and deuterated cabergoline (I.S.) (b).

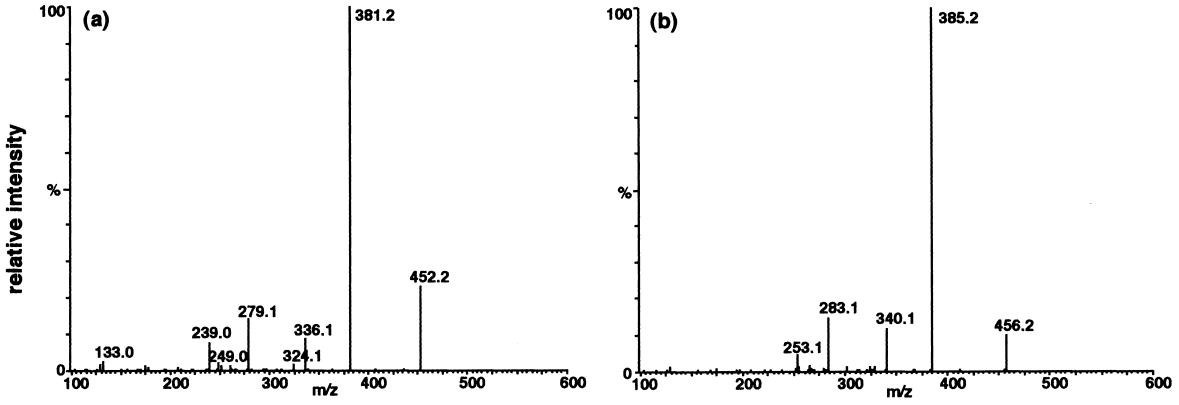

Fig. 3. Product ion spectra on LC–MS–MS of the $[M+H]^+$ ion of cabergoline (a) and deutrated cabergoline (I.S.) (b).

pretreatment of sample according to the previous method [14,19]. However, the extraction efficiency of cabergoline to organic solvent was not as much (about 52% at 20 pg/ml), and its method could not give good results on the precision of the measurements (data not shown). Therefore, we modified the preparation method of plasma samples to inject into the LC–MS–MS system. The plasma samples were added with acetonitrile and 20 m*M* ammonium formate solution (90:10, v/v) and after mixing and centrifugation, the supernatants were directly injected into the LC–MS–MS system. The calibration curve for cabergoline using MRM was obtained by plotting the peak area ratio of cabergoline (product ion m/z 381) to I.S. (product ion m/z 385) versus the amount of cabergoline. Good linearity was observed over the concentration range examined (5–250 pg/ml for standard solution, $y=0.0051x-0.0036$, $r^2=0.9974$). The calibration curve showed little day-to-day variability in the slopes and the intercepts [coefficient of variation (C.V.), <8%]. The lower limit of detection was approximately 2 pg/ml ($S/N=4$) for standard solution.

Experiments with spiked samples resulted in a recovery of 94.8±6.4% at a concentration of 20 pg/ml of control plasma sample (data not shown). Table 1 gives the intra-day and inter-day precision (C.V.%) of the method using plasma samples of patients treated with cabergoline. The intra-day precision ranged from 3.8 to 8.7%, and the inter-day precision ranged from 6.4 to 10.5%. These results indicate good reproducibility between experiments.

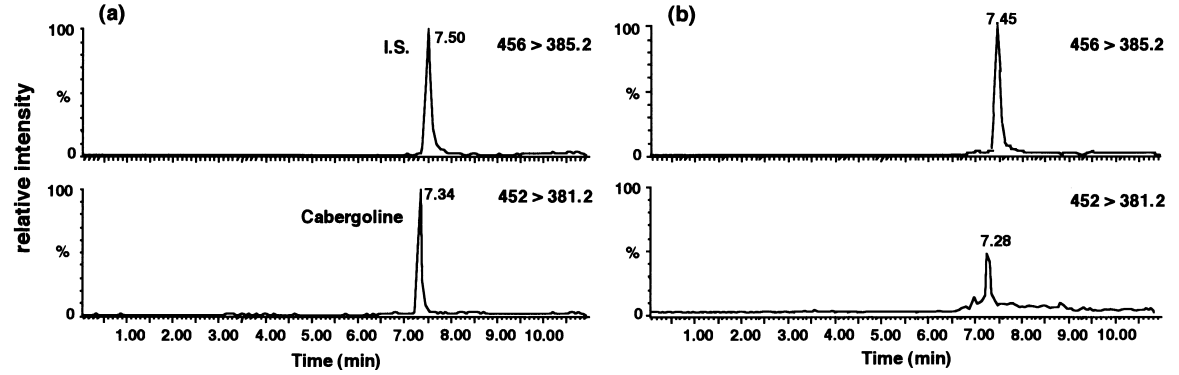

Fig. 4. MRM chromatograms on LC–MS–MS of standard solution (a) and human plasma sample. The standard solution (a) is 125 pg/ml and plasma sample obtained from patient is 15.5 pg/ml.

Table 1
Intra-day and inter-day precision of LC–MS–MS method for the determination of cabergoline in human plasma

Patient	Plasma concentration (pg/ml)	Precision (C.V.%)	
		Intra-day (n=5)	Intra-day (n=2, 4 days)
K (female, 63)	19.5±1.7	8.7	9.1
S (female, 58)	26.2±1.0	3.8	6.4
Y (male, 59)	13.8±0.7	5.1	10.5
N (female, 41)	17.5±1.4	8.0	9.8

Plasma samples were obtained from patients 1 h after oral treatment with cabergoline (0.5 mg). Plasma concentration represents mean±SD of five experiments.

We also determined L-dopa in human plasma using the same LC–MS–MS method as that of cabergoline. The ESI mass spectra of L-dopa and α-methyldopa (I.S.) showed a protonated molecule ion $(M+H)^+$ of m/z 198 and 212, respectively (Fig. 5). Moreover, ESI-MS–MS mass spectra are shown in Fig. 6. It indicates that the product ions m/z 152 of L-dopa (m/z 198) and m/z 139 of I.S. (m/z 212) are the most adequate ions for MRM of LC–MS–MS. We examined the quantitative determination of L-dopa using MRM with these ions. Without the use of liquid–liquid extraction, the plasma samples were simply treated with perchloric acid solution. After removing the plasma protein, it was diluted with HPLC mobile and then injected directly into the LC–MS–MS system. As shown in Fig. 7, the MRM chromatogram was free of interference at the retention times of interest. The calibration curve plotted the peak area ratio of L-dopa (product ion

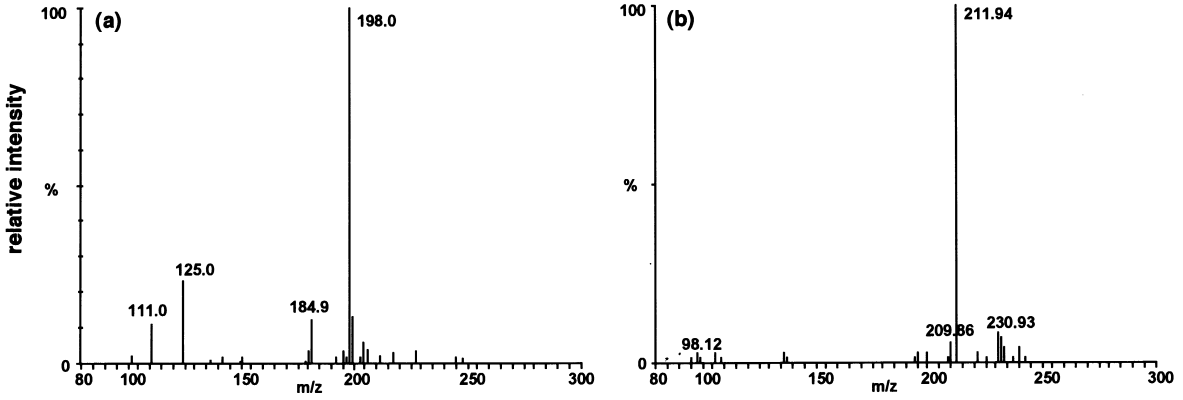

Fig. 5. LC–ESI mass spectra of L-dopa (a) and α-methyldopa (I.S.) (b).

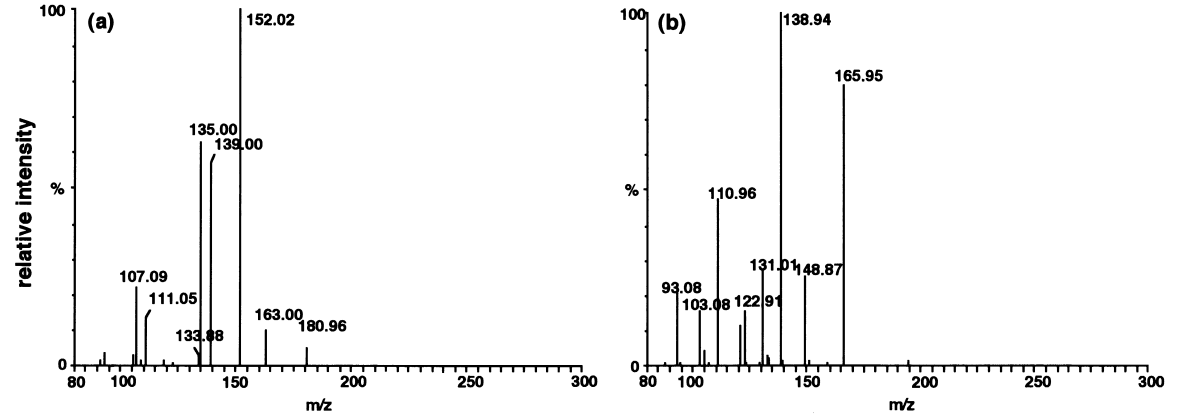

Fig. 6. Product ion spectra on LC–MS–MS of the $[M+H]^+$ ion of L-dopa (a) and α-methyldopa (I.S.) (b).

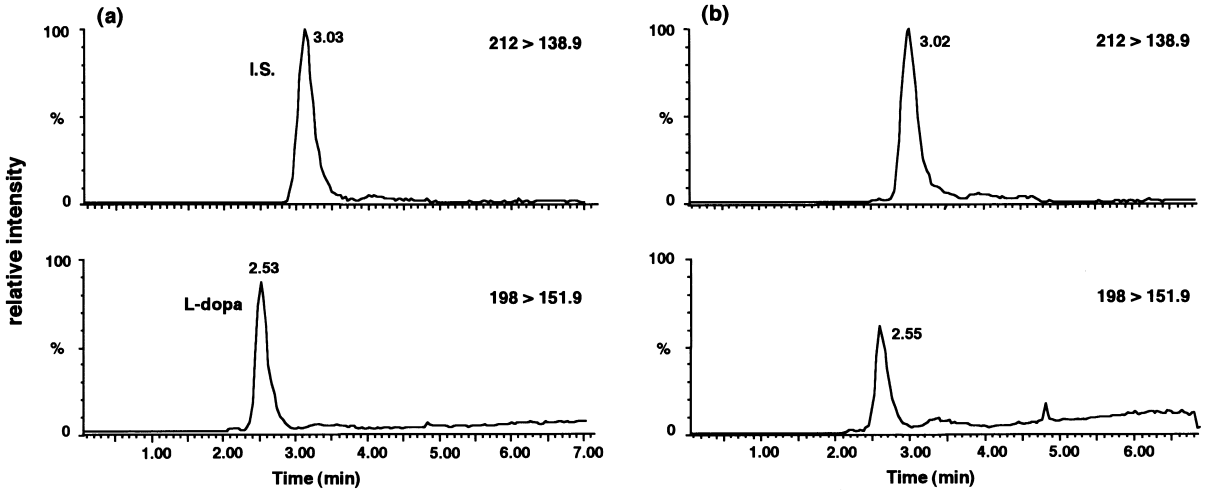

Fig. 7. MRM chromatograms on LC–MS–MS of standard solution (a) and human plasma sample. The standard solution (a) is 800 ng/ml and plasma sample obtained from the patient is 285.5 ng/ml.

m/z 152) to I.S. (product ion m/z 139) against the L-dopa concentration (ng/ml) showed a good linearity over the range examined (1–200 ng/ml for standard solution) ($y=0.1328x+0.0207$, $r^2=0.9981$). The lower limit of detection was approximately 0.1 ng/ml ($S/N=4$) for standard solution. Furthermore, the intra-day and inter-day precision (C.V.%) of the method using plasma samples of patients treated with L-dopa are shown in Table 2. The intra-day precision ranged from 2.9 to 6.4%, and the inter-day precision ranged from 6.5 to 8.1%. These results indicate good reproducibility between experiments.

We have developed a simple determination for cabergoline and L-dopa using an LC–MS–MS system. In the previous methods, sample preparations for LC, for example, liquid–liquid extraction for cabergoline [14,19] and purification by alumina for L-dopa [18] were complex and took a lot of time, and good reproducibility could not be obtained. In order to improve these problems, we examined the direct analysis by MRM method on LC–MS–MS without liquid–liquid extraction. After injection of the sample into the system, the delay of sample introduction to the mass spectrometer by use of a switching valve could be protected from the interference of ionization and increase the ion intensity. The present procedure demonstrated that this technique was simple, had good reproducibility and was very useful for routine analysis of cabergoline and L-dopa.

Table 2
Intra-day and inter-day precision of LC–MS–MS method for the determination of L-dopa in human plasma

Sample	Plasma concentration (pg/ml)	Precision (C.V.%)	
		Intra-day ($n=5$)	Intra-day ($n=2$, 4 days)
S (female, age 58)			
(CG-105, 0.25 mg)	405.2+11.7	2.9	6.5
(CG-105, 0.5 mg)	522.1+32.9	6.3	8.1
(CG-105, 1.0 mg)	302.5+19.4	6.4	8.9

Plasma samples obtained from patients 1 h after oral treatment with L-dopa (200 mg) and cabergoline (CG-105, 0.25–1.0 mg) plasma concentration represents mean±S.D of five experiments.

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