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Short communication

The effect of Madopar on the pharmacokinetics of ropinirole in healthy Chinese volunteers

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Abstract

Ropinirole is a nonergoline dopamine D_2-receptor agonist and has been proven to be effective in both monotherapy and combination therapy for idiopathic Parkinson's disease. The purpose of the present study was to examine the effect of Madopar on the pharmacokinetics of ropinirole in healthy Chinese volunteers by using liquid chromatography tandem mass spectrometry (HPLC/MS/MS). A single dose of 1 mg ropinirole was given orally after administration of the placebo or Madopar (containing 200 mg levodopa and 50 mg benserazide) to six healthy males and six healthy females in a cross-over randomized study with a minimum washout period of 8 days. Pharmacokinetic parameters were calculated for both treatments. Coadministration of ropinirole and Madopar did not result in a notable change in rate or extent of availability of ropinirole, as shown by the ratios (90% confidence intervals) of 1.045 (0.900, 1.222) for C_{max} (maximum plasma concentration) and 1.167 (1.086, 1.262) for AUC_{0-inf} (the area under the concentration–time curve). Likewise, no significant difference in any of the other pharmacokinetic parameters [T_{max} (the time needed to reach the C_{max}), MRT (mean residence time), volume of distribution (V/F), and clearance (CL/F)] was observed between the treatment groups. No clinically relevant adverse effects were detected under either conditions and there are no pharmacokinetic grounds for adjusting the dose of ropinirole when given in combination with Madopar in Chinese patients.

Keywords: Ropinirole; Madopar; Levodopa; Benserazide; Pharmacokinetics; HPLC/MS/MS

1. Introduction

Parkinson's disease (PD) is one of the most common neurodegenerative disorders in humans. Pharmacotherapy for PD centers on the use of dopaminergic drugs, primarily the dopamine receptor agonists and the dopamine precursor levodopa. Madopar contains the active ingredients levodopa and benserazide hydrochloride (inhibitor of DOPA decarboxylase), sometimes known in combination as co-beneldopa with ropinirole. As a nonergoline dopamine D_2-receptor agonist, ropinirole known as 4-[2-(dipropylamino)ethyl]-1,3-dihydro-2H-indol-2-one, is indicated for the treatment of PD [1] and has been proven to be effective in both monotherapy and combination therapy for idiopathic PD. In addition to PD, ropinirole has also been used successfully in the treatment of restless legs syndrome [2] and psychosis [3]. Ropinirole and levodopa are both inactivated by liver metabolism. The principal metabolic enzyme for ropinirole is the cytochrome P450 (CYP) isoenzyme CYP1A2. Genetic variation in this isoenzyme has been surveyed in an ethnically diverse population [4]. Research about the disposition and metabolic fate of ropinirole has been carried out in animals and humans [5]. Previous work has examined the pharmacokinetics of ropinirole in Caucasian [6–8] and has observed the lack of a pharmacokinetic interaction between ropinirole and levodopa plus carbidopa at steady state in Caucasian patients with PD [9]. However the effects of Madopar on the pharmacokinetics of ropinirole in Asians have not yet been examined. The present study investigated the effect of Madopar on the pharmacokinetics of ropinirole in healthy Chinese volunteers.

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2. Experimental

2.1. Chemicals and drug

Ropinirole reference standard was a gift from Chongqing Mulberry Field Pharmaceutical Co. Ltd., China. Internal standard, diphenhydramine (IS) was supplied by National Institute for the Control of Pharmaceutical and Biological Products, China. HPLC grade water, methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). HPLC grade ammonium acetate was delivered by Tedia (USA). Both sodium dihydrogen phosphate and dibasic sodium phosphate were analytic grade and delivered by Sinopharm Chemical Reagent Co. Ltd., China.

Ropinirole tablets were supplied by Chongqing Mulberry Field Pharmaceutical Co. Ltd., China and Madopar tablets were obtained from Shanghai Roche Pharmaceuticals Ltd., China. All blank plasma samples were supplied by the Xijing Hospital, Fourth Military Medical University, China.

2.2. Instruments and methods

A liquid chromatography–tandem mass spectrometric method was established to determine ropinirole in plasma [10]. A surveyor HPLC system (Agilent, USA) with a G1397 degasser, a G1311A quaternary pump and a G1316A column oven was coupled with a API 4000 triple-quadruple spectrometer equipped with a turbo ionspray (Applied Biosystems Ltd., USA). The HPLC column used was Zorbax Eclipse XDB C-18 (150 mm × 4.6 mm ID, 5 μm) purchased from Agilent Ltd., USA. The guard column used was (4 mm × 3.0 mm ID, 5 μm) purchased from Phenomenex Inc., USA.

The mobile phase consisted of acetonitrile–ammonium acetate (5 mM)–methanoic acid (50:50:0.1, v/v/v) at a flow rate of 0.5 ml/min through the analytical column. The mass spectrometer was operated in the electrospray ionization mode with positive ion detection. The electrospray ion temperature was maintained at 450 °C and a voltage of 4200 V was applied to the sprayer needle. Nitrogen was used as the ion spray gas (50 psi for ropinirole; 60 psi for IS) and sheath gas (10 psi). The collision energy for both ropinirole and IS was 27 eV. The analytes were monitored by multiple reaction monitoring (MRM) of the transition *m/z* 216 → *m/z* 160 for ropinirole and *m/z* 256 → *m/z* 167 for IS (Fig. 1).

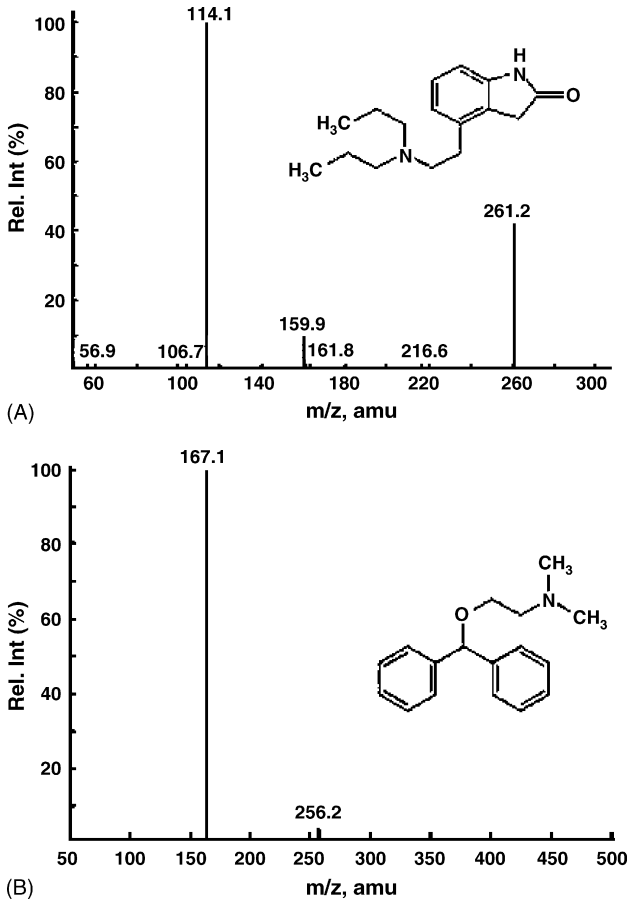

Fig. 1. Electrospray product ion mass spectrum of the precursor ion of ropinirole (A) and IS diphenhydramine (B). The analytes were monitored by multiple reaction monitoring (MRM) of the transition *m/z* 216 → *m/z* 160 for ropinirole and *m/z* 256 → *m/z* 167 for IS.

2.3. Analytical procedure

2.3.1. Preparation of stock solutions, calibration standard and quality control samples

The stock solution of ropinirole was prepared in methanol at concentration of 1.0 mg/ml. The stock solution of IS was prepared in methanol at a concentration of 100 μg/ml and diluted to 10 ng/ml with methanol. Ropinirole stock solution of 1.0 mg/ml was used to prepare the working solutions for preparation of the seven-point calibration curve (10.0, 35.0, 100, 350, 800, 2000, 5000 pg/ml) and quality control samples (QC) at three concentration levels (30.0, 300, 4500 pg/ml). All stock solutions were kept refrigerated 4 °C when not in use.

2.3.2. Collection and preparation of the samples

The following study protocol was approved by the Ethics Committee of the Xijing Hospital, Fourth Military Medical University, China. The volunteers gave their written informed consent before entering the study. A randomized cross-over study design with two phases was used with an interval of 8 days. Twelve healthy volunteers (six men, six women; age range, 27–28 years; height range, 162–172 cm; body mass index, 19–24) participated in the study. Each person was found to be in good health, as determined by a medical history, clinical examination, and routine laboratory tests. Either 1 mg ropinirole (two ropinirole 0.5 mg tablets) plus placebo or 1 mg ropinirole plus Madopar (Madopar, one tablet containing 200 mg levodopa and 50 mg benserazide) were administered with 200 ml water at 8 a.m. Timed blood samples (5 ml) were drawn from a forearm vein into heparinized tubes before administration of ropinirole and at 0.5, 0.75, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 24, and 36 h later. Plasma samples were separated within 10 min (3500 rpm) and stored at −20 °C until analyzed.

A 100 μl aliquot of sample was pipetted into a microtube. The following were then added to the tube: 50 μl of methanol–water (50:50, v/v), 50 μl of IS (10.0 ng/ml) and 200 μl phosphate buffer (0.5 mol/ml, pH 12). After being vortexed for 1 min and vigorously mixed for 10 min (240 times/min) with a rotating mixer, the sample mixture was centrifuged for 5 min (3500 rpm) and the organic phase was transferred to another clean tube and evaporated under air stream at 40 °C. The residue was reconstituted with a 150 μl aliquot of mobile phase. A 20 μl aliquot of mobile phase was injected into the HPLC column.

2.4. Pharmacokinetic analysis

The non-compartmental model analysis was used in the data processing of ropinirole. C_{max} and T_{max} were determined by inspection of the plasma concentration–time curves. K_e was determined by linear regression of the terminal linear portion of the concentration–time curve, and $T_{1/2}$ was calculated as $\ln(2)/K_e$. AUC was calculated by the linear trapezoidal rule. Clearance (CL/F) was calculated as dose/AUC_{inf}.

3. Result and discussion

3.1. Validation of the method

Good selectivity was observed, and there was no significant interference or ion suppression from endogenous substances observed at the retention time of the analytes (Fig. 2). Typical retention times for ropinirole and IS were about 2.60 and 3.35 min, respectively.

The peak area ratios of ropinirole to IS in human plasma were linear with respect to the analyte concentrations over the range of 10–5000 pg/ml. The calibration curve was fit by a least-square $1/\chi^2$-weighted linear regression method and the average slope and intercept of regression equations were 6.441×10^{-4}

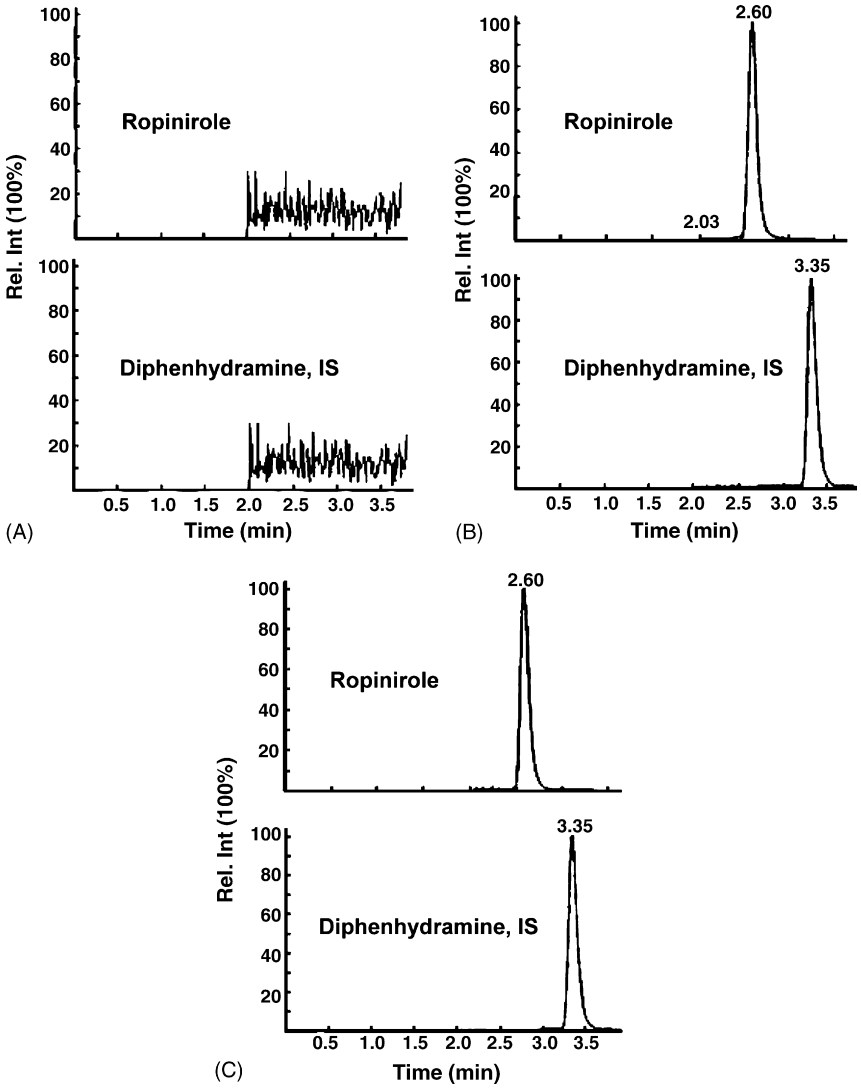

Fig. 2. Representative chromatograms obtained from human plasma samples: (A) blank human plasma; (B) plasma sample spiked with ropinirole at 800 pg/ml and diphenhydramine (IS) at 5 ng/ml; (C) a volunteer 1 h after receiving 1 mg oral dose of ropinirole.

Table 1
The pharmacokinetic variables of ropinirole in 12 healthy volunteers after administration of 1 mg ropinirole following ingestion of 1 dose Madopar (200 mg levodopa and 50 mg benserazide) or placebo (mean ± S.D.)

Variable	Placebo	Madopar	*P*-Value	Geometric mean ratio (Madopar/placebo)	90% CI
C_{max} (pg/ml)	1559 ± 508	1629 ± 494	0.49	104.5	90.0–122.2
T_{max} (h)	1.40 ± 0.53	1.23 ± 0.49	0.40		
$T_{1/2}$ (h)	6.67 ± 1.13	6.57 ± 1.11	0.89		
$AUC_{0-36\,h}$ (ng/h/ml)	10.8 ± 3.1	12.6 ± 3.1	0.002	116.7	108.6–126.2
AUC_{0-inf} (ng/h/ml)	11.1 ± 3.4	12.9 ± 3.1	0.003	116.2	108.5–126.3
V/F (l)	916 ± 256	771 ± 201	0.48		
CL/F (l/h)	95.7 ± 21.8	81.2 ± 16.3	0.33		
MRT (h)	8.51 ± 1.12	8.49 ± 1.05	0.92		

and 1.092×10^{-2}, and correlation coefficients were consistently greater than 0.99. The limit of quantification (LOQ), defined as the lowest concentration on the standard curve that can be measured with acceptable accuracy and precision (<20%), was established at 10 pg/ml (signal-to-noise, S/N ≥ 10).

The precision and accuracy of the method were described as relative standard deviation (R.S.D.%) and relative error (RE%), respectively. The intra-run R.S.D. and inter-run R.S.D. of ropinirole were 7.3% and 6.5%, respectively (30.0, 300.0, 4500.0 pg/ml; $n = 6$). The accuracy of ropinirole, determined at three concentrations (30.0, 300.0, 4500.0 pg/ml; $n = 6$) was within 2.0%. The recovery of ropinirole (30.0, 300, 4500.0 pg/ml; $n = 6$) was 82.4 ± 3.9%, 75.6 ± 5.0%, and 70.4 ± 2.9%, respectively. The recovery of IS was 79.8 ± 2.1% ($n = 6$).

The stability of ropinirole and IS under different storage conditions was evaluated. The stock solutions of ropinirole and IS were stable at room temperature for more than 8 h and at 4 °C for a month. The accuracy values of ropinirole (30.0, 4500 pg/ml; $n = 6$) were 1.7% and −5.4% after three freeze-thaw cycles, and −0.9% and −4.1% at −20 °C for 30 days, respectively. The accuracy values of processed ropinirole samples (30.0, 4500.0 pg/ml; $n = 6$) were 1.7% and −5.4%, respectively, when kept at room temperature for 24 h.

3.2. Pharmacokinetics

Fig. 3 shows the mean plasma concentration–time curve of ropinirole, Table 1 provides its mean pharmacokinetic parameters and range. Ropinirole had approximately linear pharmacokinetics when given as a single dose, and was eliminated with a half-life of approximately 6 h. Peak plasma concentrations of ropinirole were reached within 0.74–2 h. These findings are in agreement with the previously reported pharmacokinetic parameters of ropinirole [6–9]. Although the mean AUC_{inf} and C_{max} of ropinirole were 16.2% and 8.9% greater respectively in the Madopar phase than in the placebo phase, the other pharmacokinetic parameters were not significantly different between the two phases. This suggests that Madopar has no statistically significant effect on the pharmacokinetic variables of ropinirole in healthy Chinese volunteers.

Previous studies have examined other drugs that were frequently administered with ropinirole and are likely to influence ropinirole's pharmacokinetics, as they are substrates or inhibitors of the CYP1A2 and CYP3A enzymes that metabolize ropinirole [11]. However, domperidone [12], theophylline [13] and the tricyclic antidepressants [14] have little-to-no effect on the pharmacokinetics of ropinirole when coadministered with ropinirole.

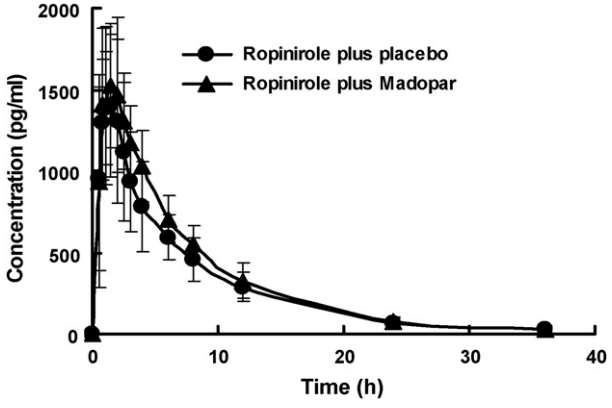

Fig. 3. Plasma concentration–time curve of ropinirole in 12 healthy Chinese volunteers after administration of 1 mg ropinirole following ingestion of one dose Madopar or placebo (mean ± S.D.).

4. Conclusions

The present findings suggest that ropinirole is well tolerated in Chinese patients when given alone or in combination with Madopar. Thus, there is no sufficient pharmacokinetic reason to adjust the dose of ropinirole when it is coadministered with Madopar in Chinese patients.

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