Determination of ornidazole in plasma by HPLC and study on its pharmacokinetics and bioequivalence of domestic and imported tablets in 18 healthy volunteers

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ABSTRACT: OBJECTIVE To establish a reversed HPLC method for the determination of ornidazole in human plasma, and study the pharmacokinetics and bioequivalence of domestic and imported tablets in healthy volunteers. **METHOD** The drug was extracted from plasma with methanol and isopropanol (50∶50), and then analyzed by HPLC with a C_{18} 5μm 250 × 4.6mm column and UV detector set at 316nm. The mixture of methanol and 0.4% glacial acetic acid (50∶50) was used as the mobile phase. The flow rate is 0.8mL·min^{-1}. 1.5g single oral dose of domestic and imported ornidazole tablets were given to 18 healthy volunteers in an open randomized crossover study. The pharmacokinetic parameters as well as relative bioavailability were measured. **RESULTS** The calibration curve was linear within the range of 2.0 ~ 20.0μg·mL^{-1} and the measurable lowest limit was 0.2μg·mL^{-1}. The average recovery of ornidazole at the concentrations of 2.0, 10.0, 20.0μg·mL^{-1} was 100.36%, 98.21% and 97.42%, respectively. The RSD of the within-day and between-day were less than 7% and 6%, respectively. The results showed that the concentration-time curves of the two preparations were fitted to a two-compartment model with a lag time. The major pharmacokinetic parameters of domestic and imported ornidazole tablets were shown respectively as following: $t_{1/2(\beta)}$ were (16.29 ±2.20) h and (15.85 ±2.26) h; T_{max} were (1.67 ±0.49) h and (1.75 ±0.48) h; C_{max} were (22.03 ±3.53) mg·L^{-1} and (22.58 ±5.94) mg·L^{-1}; $AUC_{0\sim72}$ were (444.56 ±55.87) mg·h·L^{-1} and (433.31 ±58.52) mg·h·L^{-1}; $AUC_{0\sim\infty}$ were (462.95 ±55.35) mg·h·L^{-1} and (451.67 ±57.97) mg·h·L^{-1}. There were no significant difference between the main pharmacokinetic parameters of the two preparations ($P > 0.05$). The relative bioavailability of domestic tablet was (102.91 ±8.93)%. **CONCLUSIONS** This HPLC method is simple, sensitive and accurate. It is suitable for routine determination of ordinazole levels in human plasma and for its pharmacokinetic study. The result of the statistical analysis showed that the two preparations were bioequivalent.

KEY WORDS: ornidazole; HPLC; pharmacokinetics; bioequivalence

奥硝唑血药浓度的高效液相色谱法测定及其国产和进口片剂的药动学和生物等效性研究

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摘要:目的　建立血浆中奥硝唑浓度的反相高效液相色谱分析方法,并用此法研究了国产和进口奥硝唑片剂在健康人体内的药动学及生物等效性。方法　用甲醇/异丙醇(50/50)提取样本,采用 Kromasil C_{18}色谱柱,以甲醇∶0.4%HAc(50∶50)为流动相,流速 0.8mL·min^{-1},紫外检测波长 316nm。18 名健康男性志愿者随机交叉口服国产及进口奥硝唑片 1.5g,测定其药动学参数,评价两种制剂的生物等效性。结果　奥硝唑在 2.0 ~ 20.0μg·mL^{-1}范围内呈线性,r = 0.9997,最低检测限 0.2μg·mL^{-1}。低、中、高浓度(2.0,10.0,20.0μg·mL^{-1})的方法回收率分别为 100.36%,98.21%和 97.42%,日间及日内 RSD 分别 < 6%和 < 7%。药动学研究表明,口服奥硝唑国产与进口制剂的药-时曲线符合有滞后时间的二室模型。其主要药动学参数如下:$t_{1/2(\beta)}$分别为(16.29 ±2.20) h 和(15.85 ±2.26) h;T_{max}分别为(1.67 ±0.49) h 和(1.75 ±0.48) h;C_{max}分别为(22.03 ±3.53) mg·L^{-1}和(22.58 ±5.94) mg·L^{-1};$AUC_{0\sim72}$分别为(444.56 ±55.87) mg·h·L^{-1}和(433.31 ±58.52) mg·h·L^{-1};$AUC_{0\sim\infty}$分别为(462.95 ±55.35) mg·h·L^{-1}

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和(451.67±57.97) mg·h·L^{-1}。两种制剂主要药动学参数均无显著性差异($P>0.05$)。国产奥硝唑片的相对生物利用度为(102.91±8.93)%。结论　本法准确可靠,操作简便,适用于临床药动学研究及常规血药浓度监测。统计学检验结果提示,国产和进口奥硝唑片具有生物等效性。

关键词:奥硝唑;高效液相色谱法;药动学;生物等效性
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Ornidazole [α-(chloromethyl)-2-methyl-5-nitroimidazole-1-ethanol] is a derivative of nitroimidazole, with antiprotozoal and antibacterial properties. It is used in the prevention and treatment of infections due to anaerobic germ, such as Bacteroides fragilis. The drug is especially useful in abdominal or gynecological surgery.

The aim of this study was to establish a reversed high performance liquid chromatography (HPLC) for the determination of ornidazole in human plasma and to study the pharmacokinetics and bioequivalence of domestic and imported ornidazole tablets in 18 Chinese healthy male volunteers by oral administration.

1　Materials and Methods

1.1　Drug and reagents

Drugs: Domestic ornidazole tablet was provided by Huamei Institute of Medicine Research in Changsha (250 mg/Tab, Lot No: 20000418); imported ornidazole tablet called Tiberal ROCHE was provided by ROCHE PRODUCTS, NEW ZEALAND LTD AUCKLAND (500 mg/Tab, Lot No: B1003MFD08, 1998); ornidazole standard (Lot No: 000401, purity: 99.8%) and the internal standard α-(ethoxymethyl)-2-nitroimidazole-1-ethanol were provided by Huamei Institute of Medicine Research in Changsha.

Reagents: Methanol was HPLC-grade. Isopropanol and glacial acetic acid were analytical grade. Water was redistilled.

Standard solution: The suitable ornidazole standard was dissolved with methanol to 100 μg·mL^{-1}. The suitable internal standard was dissolved with methanol to 100 μg·mL^{-1}.

1.2　Chromatography condition

The following modular HPLC system was used: 125 HPLC pump (Beckman, USA), 166 UV detector (Beckman, USA). The analytical column (250 mm×4.6 mm) was packed with Kromasil C_{18} (5 μm). The mobile phase was composed of methanol and 0.4% glacial acetic acid (50∶50). The flow rate was 0.8 mL·min^{-1}, and the monitoring UV wavelength was 316 nm.

1.3　Subjects

18 healthy male volunteers with free consent were aged (22.39±1.24) a, weighed (64.83±7.96) kg, and all the test results of their blood, urine, liver, kidney, and electrocardiogram were within normal ranges.

1.4　Study design

After 12 h of overnight fasting, the 18 volunteers received a single oral dose of 1.5 g ornidazole tablets either domestic or imported (9 for domestic tablets and 9 for imported tablets) in an open randomized crossover study design. A uniform diet was supplied after 4 h and water 200 mL was drunk. Each dosing was followed by a washout period of 1 wk before the next administration.

1.5　Plasma collection

Blood samples (4 mL) were collected into the heparinized tubes before administration and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, 48, 72 h after administration. The samples were centrifuged immediately at 3 000 r·min^{-1} for 5 min and the separated plasma were stored at -20 ℃ until analyzed.

1.6　Sample preparation

Frozen samples were allowed to thaw and warm to room temperature. After vortex-mixing, 0.5 mL of the sample was pipetted into a conical extraction tube. Then 100 μL of 100 μg·mL^{-1} internal standard solution and 2.0 mL methanol-isopropanol (50∶50) were added. The mixture was agitated for 2 min using a vortex agitator and then centrifuged for 10 min at 2000 r·min^{-1}. Then 10 μL of supernatant were injected into the chromatograph.

1.7　Establishment of standard curves

A standard concentration curve was obtained by adding ornidazole standard solution at concentrations of 2.0, 5.0, 10.0, 15.0, 20.0 μg·mL^{-1} to blank human plasma under the same experimental conditions. The ratios of the peak areas of ornidazole and internal standard were plotted. Calibration curve was linear over the range of 2.0~20.0 μg·mL^{-1}. A linear equation was $Y=0.0508X+0.0023$ ($r=0.9997$). The minimal detect limit of plasma ornidazole was 0.2 μg·mL^{-1}.

1.8　Reproducibility and recovery

To test the within-day and between-day reproducibility, 5 aliquots of each sample were assayed within 1 d and 5 d at drug levels of 2.0, 10.0, 20.0 μg·mL^{-1}. The results were showed in the Tab 1.

5 aliquots of each sample were assayed at drug levels of 2.0, 10.0, 20.0 μg·mL^{-1} to test the recovery. The results were shown in the Tab 1.

Tab 1　Reproducibility and recovery of ornidazole in plasma by HPLC ($n=5$)

表 1　HPLC 法测定血浆中奥哨唑的重复性及回收率

Added (mg·L^{-1})	Within-day RSD (%)	Between-day RSD (%)	Recovery (%)
2.0	3.29	5.43	100.36±1.69
10.0	6.61	0.69	98.21±1.36
20.0	1.27	0.94	97.42±1.35

1.9　Data analysis

Compartments model of ornidazole plasma concentrations were fitted and then pharmacokinetic parameters were calculated with

3P97 program which was compiled by Chinese Association of Mathematical Pharmacology on a Pentium computer. Tmax and Cmax were the measured values. To determine if the domestic and imported ornidazole tablets have the equivalent biological effects, the parameters including $t_{1/2(\beta)}$, Tmax, C_{max}, $AUC_{0\sim72}$ and $AUC_{0\sim\infty}$ were analyzed with ANOVA and two one-sided t tests. The relative bioavailability of domestic tablet was calculated by the following formula.

The relative bioavailability of domestic tablet = $AUC_{0\sim\infty}$(domestic) / $AUC_{0\sim\infty}$(imported) × 100 %

2 Results

2.1 HPLC chromatograms of ornidazole

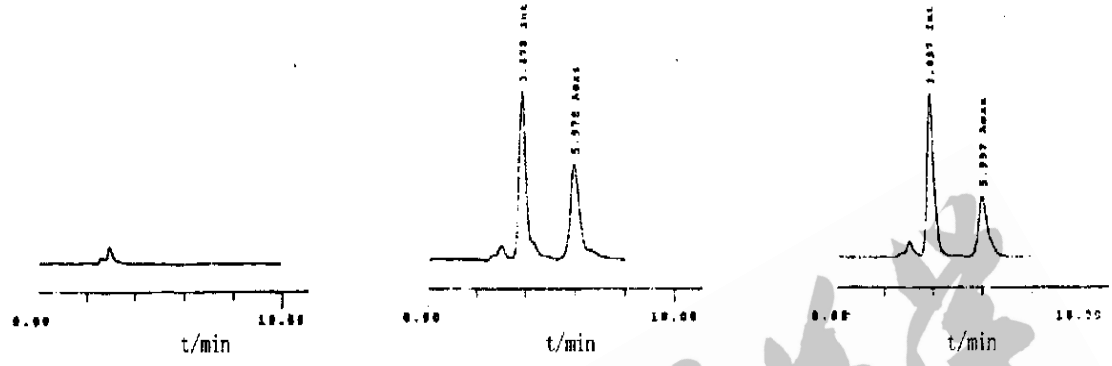

Fig 1 HPLC chromatograms of ornidazole

图 1 奥哨唑 HPLC 图谱

A. blank plasma; B. ornidazole standard; C. plasma sample

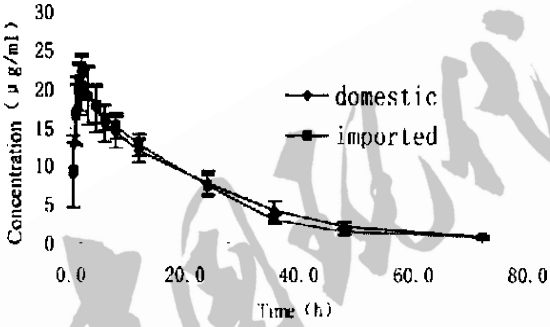

Fig 2 Mean plasma concentration-time curves of ornidazole after a single oral dose of 1.5g in 18 volunteers

图 2 18 位志愿者单次口服 1.5g 奥哨唑后的血药浓度-时间曲线

2.2 Pharmacokinetics

The changes in plasma concentration were best described by two-compartment model with a lag time. The mean plasma concentration-time curves of ornidazole were shown in Fig 2. The major pharmacokinetic parameters were given in Tab 2.

Tab 2 The major pharmacokinetic parameters of ornidazole after a single oral dose of 1.5g (n = 18)

表 2 单次口服 1.5g 奥哨唑后的主要药动学参数

Parameters	Domestic	Imported
$t_{1/2}(\beta)$ (h)	16.29 ± 2.20	15.85 ± 2.26
T_{max} (h)	1.67 ± 0.49	1.75 ± 0.48
C_{max} (μg/mL)	22.03 ± 3.53	22.58 ± 5.94
$AUC_{0\sim72}$ (μg/mL·h)	444.56 ± 55.87	433.31 ± 58.52
$AUC_{0\sim\infty}$ (μg/mL·h)	462.95 ± 55.35	451.67 ± 57.97

2.3 Equivalence of domestic ornidazole tablet

The multi-factorial analysis of variance for data of $t_{1/2(\beta)}$, T_{max}, C_{max}, $AUC_{0\sim72}$ and $AUC_{0\sim\infty}$ were given in Tab 3, which revealed that all of t_1 and t_2 were larger than the critical value ($t_{1-\alpha}$ = 1.746). So the domestic and imported ornidazole tablets were bioequivalent. The relative bioavailability of domestic tablet was (102.91 ± 8.93) % as compared to imported one.

Tab 3 The results of two one-side t-test

表 3 双单侧 t 检验结果

parameters	t_1	t_2	$t_{(1-0.05)}$
$AUC_{0\sim\infty}$	13.646	10.847	1.746
$AUC_{0\sim72}$	12.452	9.797	1.746
T_{max}	9.873	9.549	1.746
$T_{1/2}$	7.925	6.236	1.746
C_{max}	19.086	21.423	1.746

3 Discussion

Determination of ornidazole in human plasma by HPLC has not been reported in China. According to the oversea essays[1,2], this HPLC method was established by improving chromatography condition and changing the mobile phase. The extraction was more efficient and the examination step was simpler. This HPLC method is rapid and reproducible. The selectivity is good and its sensitivity is sufficient for pharmacokinetic studies and therapeutic drug monitoring.

The result of the study on pharmacokinetics showed that the plasma concentration-time curves of the domestic and imported ornidazole tablets were all fitted to two-compartment model with a lag time. There were no significant difference between the two preparations in absorption, distribution and elimination. The major pharmacokinetic parameters of domestic and imported ornidazole tablets in

this study were similar to those in oversea essays[3,4].

According to the ANOVA, there was no difference between the 1st and 2nd medications in the randomized crossover study. The bioequivalence analysis also showed that there was no significant difference between the two preparations, so domestic and imported ornidazole tablets were bioequivalent.

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