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ORIGINAL RESEARCH

Bioequivalence study of two formulations of flupirtine maleate capsules in healthy male Chinese volunteers under fasting and fed conditions

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Aim: This study developed a high-performance liquid chromatography–tandem mass spectrometry method to simultaneously determine the concentrations of flupirtine and its major active metabolite D-13223 in human plasma in order to assess the bioequivalence (BE) of two flupirtine maleate capsules among healthy male Chinese volunteers under fasting and fed conditions.

Materials and methods: There were two single-center, randomized, single-dose, open-label, laboratory-blinded, two-period, cross-over studies which included 24 healthy male Chinese volunteers under fasting and fed conditions, respectively. Plasma samples were collected prior to and up to 48 h after dosing. The concentrations of flupirtine and its major active metabolite D-13223 in plasma samples were determined by a validated method, that is, high-performance liquid chromatography coupled with a tandem mass spectrometry detector. Pharmacokinetic metrics of area from time zero to the last measurable concentration (AUC_{0-t}), area under the plasma concentration–time curve from administration to infinite time ($AUC_{0-\infty}$), and C_{max} were used for BE assessment.

Results: Forty-eight healthy volunteers who met the criteria were enrolled and completed the study. According to the observation of vital signs and laboratory measurement, no volunteers had any adverse reactions. Under fasting condition, the geometric mean ratios (90% CI) of the test/reference drug for flupirtine were 103.0% (98.1%–108.2%) for AUC_{0-t}, 102.9% (98.2%–107.9%) for $AUC_{0-\infty}$, and 97.0% (85.9%–109.5%) for C_{max}. Under fed condition, the geometric mean ratios (90% CI) of the test/reference drug for flupirtine were 101.7% (98.4%–105.1%) for AUC_{0-t}, 101.6% (98.5%–104.8%) for $AUC_{0-\infty}$, and 103.5% (94.7%–113.0%) for C_{max}. The difference between test and reference formulations, T_{max}, was not statistically significant. The 90% CIs of the test/reference AUC ratio and C_{max} ratio of D-13223 were also within the acceptance range for BE both under fasting and fed conditions.

Conclusion: The two formulations of flupirtine maleate capsule were bioequivalent (the test and the reference products) under fasting and fed conditions, and thus both can be used interchangeably in the clinical setting.

Keywords: flupirtine, D-13223, LC–MS/MS

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Introduction

Flupirtine, ethyl-*N*-[2-amino-6-(4-fluoro-phenylmethyl-amino)pyridine-3-yl] carbamate, is a non-opioid analgesic without antipyretic or antiphlogistic properties. It acts centrally on γ-aminobutyric acid A receptors and the selective neuronal Kv7 potassium channel,[1–3] thus offering a mechanism-based therapy for pain relief and normalization of muscle tension.[4,5]

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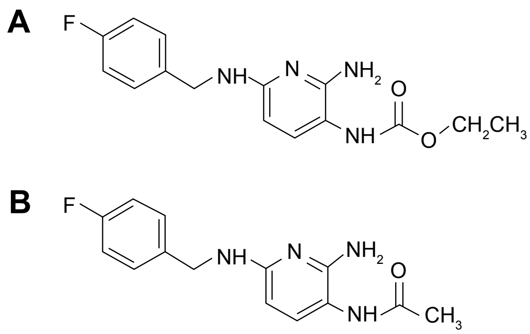

Figure 1 Flupirtine (**A**) and D-13223 (**B**).

In patients with acute and chronic pain, flupirtine is clinically as efficient as weak opioids and NSAIDs,[4–7] and its well-known neuroprotective properties make this drug a possible candidate for the treatment of Parkinson's, Alzheimer's, and Creutzfeldt–Jakob's disease and other neurodegenerative disorders.[8–11] Clinical interest in flupirtine is growing in recent years.

As reported, flupirtine is a hydrophilic compound. It is completely absorbed from gastrointestinal tract with a bioavailability of 90% by oral route.[12] One hundred milligrams of oral flupirtine in normal healthy volunteers reached a peak plasma concentration (C_{max}) of 0.773 μg/mL at 1.6 h. It gets equally distributed into both extra- and intravascular compartments. The apparent volume of distribution is 154 L, half-life is 6.5 h, and the clearance is 275 mL/min. Flupirtine is metabolized in liver to 4-fluorohippuric and *N*-acetylated analog D-13223 (Figure 1) by peroxidase enzymes. The *N*-acetylated metabolite D-13223 retains 20%–30% of active parent compound. The two metabolites are further oxidized and then conjugated with glycine to form inactive metabolites.[6,13] Seventy-two percent of the total dose administered appears in urine as parent drug and its metabolites, whereas 18% is excreted in feces.[14]

Flupirtine maleate capsule (Katadolon®; AWD LTD, Hannover, Germany) has been effectively and safely used in Germany for 20 years. However, its use was limited due to its high price and poor cognition in China. The aim of this study was to develop a high-performance liquid chromatography coupled with a tandem mass spectrometry detector (LC–MS/MS) method to simultaneously determine the plasma flupirtine and its major active metabolite D-13223 concentrations, and thereby to assess bioequivalence (BE) for the same dose (100 mg) of two flupirtine formulations (generic drug and Katadolon) among healthy male Chinese volunteers under fasting and fed conditions.

Materials and methods

Study protocol

Ethics committee of General Hospital of Shenyang Military Area Command approved the clinical study protocol and a no-objection letter was obtained from the China Food and Drug Administration. The study was performed in accordance with the Helsinki Declaration and Good Clinical Practice Guideline.

Volunteers

Forty-eight volunteers were judged eligible for enrollment by compliance with all the inclusion and exclusion criteria described in the protocol. The criteria were as follows: male, healthy, adult non-smokers or ex-smokers, aged 18–40 years with body mass indices 19–25 kg/m^2, clinically significant diseases were not captured in the medical history, and no evidence of clinically significant findings on physical examination and/or clinical laboratory evaluations (hematology, blood biochemistry, hepatic function, electrocardiogram examination, and urinalysis) was found. Participants were needed to have negative test results for human immunodeficiency virus, hepatitis B, and hepatitis C. All qualified participants signed an informed consent form prior to study commencement.

Drugs and materials

The test preparation of flupirtine maleate capsule (100 mg) was produced by the sponsor, PRC. The batch number was D150101. The reference preparation of katadolon (100 mg) was produced by AWD pharma GmbH Co.KG, LTD, Hannover, Germany. The batch number was 1384 and was provided by the sponsor.

Flupirtine maleate, D-13223, stable-isotope internal standard (IS) flupirtine-d5(F-d5) and D-13223-d4(D-d4) (purity 99.9%, 99.5%, 99.5%, and 98.0% respectively) were purchased from Toronto Research Chemicals Inc. (Toronto, Canada). Optima-grade methanol and acetonitrile were obtained from Fisher Scientific (Fairlawn, NJ, USA). All other chemicals and solvents were of analytical grade. Milli-Q water was obtained from a Millipore system (Millipore, Burlington, MA, USA).

Study design

This study consists of two independent clinical trials (fasting BE study and fed BE study), both of them were single-center, randomized, open-label, single-dose, laboratory-blinded, two-period, cross-over studies including 24 healthy male volunteers for each of the study. The same subject can participate in only one clinical trial.

Fasting BE study

Twenty-four volunteers were hospitalized in the study site the night before drug administration, and were randomized into two groups, group A and group B. After overnight fasting for at least 10 h, group A was given test capsule, group B got the reference capsule (katadolon) in the morning. After the capsules were swallowed with 200 mL water, compliance of oral dosing was supervised by mouth and drug container inspection. Standard meals were provided 4 and 10 h after administration. Drinking of water was restricted 1 h prior to and after administration. After a 7-day washout, alternate capsule was administered and the experiment was repeated.

Fed BE study

The study was conducted with another 24 volunteers. It was similar to the fasting one except that both test and reference capsules were administered following a high-fat, high-calorie meal, which consisted of 150 calories protein, 250 calories carbohydrate, and 500 calories fat. Volunteers started the recommended meal 30 min prior to administration and finished in 30 min. The standardized meals scheduled at the same time were the same in both of the two periods of the study.

Blood sampling

The venous blood samples of both fasted and fed treatments were collected at time intervals (0, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 6.0, 8.0, 12, 24, 36, and 48 h) in separate 5-mL vacuum blood collection tubes. Blood samples were centrifuged at 1,500× *g* for 10 min and the plasma was separated and kept frozen at −70°C until analysis.

Analytical method

The concentrations of flupirtine and its major active metabolite D-13223 were determined by LC–MS/MS based on the method of Chen et al[15] with an API 3200 Q-Trap mass spectrometer equipped with a TurboIonSpray Ionization source. Chromatography was performed on an Agilent ZORBAX (2.1×150 μm; 5 μm), using a mobile phase of acetonitrile–water–ammonia (55:45:0.1, v/v/v). The flow rate was 0.25 mL/min. The column temperature was maintained at 40°C. The total LC analysis time per injection was 3.6 min with isocratic elution. An injection volume of 20 μL was used for all samples.

Sample preparation

A 200 μL aliquot of human plasma was added to 20 μL ISs working solution and 20 μL mobile phase and vortex for 30 s. The sample mixture was extracted with 1 mL ether–methylene chloride (3:2, v/v) and then vortex 30 s and centrifuged at 15,000× *g* for 10 min. The supernatant was transferred to another tube and evaporated under a gentle stream of air at room temperature to dryness. The residue was reconstituted in 100 μL mobile phase and vortex-mixed for 30 s. An aliquot of 20 μL was injected into the LC–MS/MS system for analysis.

Method validation

Flupirtine and D-13223 were detected and validated simultaneously by analyzing spiked plasma samples. According to relevant guidelines,[16,17] validation included 1) selectivity, 2) accuracy and precision, 3) recovery and matrix effect, 4) the calibration curve, 5) lower limit of quantification, and 6) stability of analytes in spiked samples.

Tolerability

Tolerability assessment was primarily completed by monitoring volunteer's vital signs (blood pressure and heart rate). Time points were measured at baseline (predose) and at 2 and 48 h after administration. At baseline and after completion of the study, laboratory tests (hematology, blood biochemistry, hepatic function, and urinalysis) and electrocardiogram examination were performed.

Pharmacokinetic study

The pharmacokinetic (PK) parameters and statistical analysis were carried out by the validated statistical software WinNonlin 6.4 (Pharsight, Princeton, NJ, USA). The C_{max} and time to C_{max} (T_{max}) were obtained directly from the experimental data. The area under the plasma concentration–time curve from administration to infinite time ($AUC_{0-\infty}$) was calculated using the linear trapezoidal method: trapezoidal area from time zero to the last measurable concentration (AUC_{0-t}), extrapolated to infinite time, by addition of the area obtained from the last measurable concentration divided by the terminal elimination rate constant (β); β was estimated from the linear least-squared regression of the terminal phase of the log concentration–time profile. The apparent biological half-life ($t_{1/2}$) was calculated as 0.693/β. The parameters are represented with mean ± SD.

Statistical analysis

To evaluate the BE of the formulations, analysis of variance (ANOVA) was performed for log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. T_{max} was analyzed using a nonparametric approach. The evaluation of fixed period, sequence, and treatment effects was based on the Wilcoxon's rank-sum test. The log-transformed C_{max} ratio, AUC_{0-t} ratio, and $AUC_{0-\infty}$ ratio were obtained for both formulations. ANOVA was performed by using the *F* test. Two one-sided *t*-tests were used to obtain the probability of exceeding the limits of acceptance for BE (80.00%–125.00%). If the log-transformed ratios of C_{max} and AUC values of the two formulations were within a predetermined equivalence range, and their 90% CI *P*-value was <0.05, the two formulations would have been considered bioequivalent.

Results

Bioanalytical method validation

This study established an LC–MS/MS method for the determination of flupirtine and D-13223 in human plasma. Method specificity was demonstrated by comparing the multiple reaction monitoring chromatograms of blank samples with those of spiked samples. The retention times of flupirtine, D-13223, and the ISs were 2.71, 2.17, 2.69, and 2.15 min, respectively. No endogenous source of interference was observed at the retention time of the analytes and ISs.

The linear ranges were 0.01–2.00 µg/mL for flupirtine and 0.002–0.400 µg/mL for D-13223 in human plasma. Typical equations of calibration curves were as follows:

$$\text{Flupirtine: } y=1.07\times10^{-3}x + 1.13\times10^{-4},\ r=0.9994;$$

$$\text{D-13223: } y=4.17\times10^{-3}x + 2.29\times10^{-4},\ r=0.9985.$$

Intra/inter-day precision and accuracy were evaluated by using quality control (QC) samples at four levels in replicates (n=6) by performing complete analytical runs on the same day and on three consecutive days. The intra-day precision was <6.92% for each QC level of flupirtine and D-13223. The inter-day precision was <9.26% for each QC level of flupirtine and D-13223. The accuracy as determined from QC samples was −5.80%–3.31% for each QC level of flupirtine and D-13223, respectively.

Six blank matrices were extracted using the proposed extraction method and the standard solutions (equal to the low concentration quality control and the high concentration quality control) containing IS were spiked into the blank matrices after extraction. It indicated that the matrix effects had no practical effect on the quantification of flupirtine and D-13223.

The stability of flupirtine and D-13223 in human plasma was investigated under a variety of storage and process conditions. The results of the stability studies, summarized in Table 1, did not reveal any significant degradation under the conditions of the experiment.

Table 1 Stability of flupirtine and D-13223 in human plasma (n=3)

Storage conditions	Flupirtine		D-13223	
	20.0	**1,600**	**4.00**	**320**
Processed sample at room temperature for 6 h	17.0	1,367	3.79	297
RE %	−14.8	−14.6	−5.17	−7.19
Unprocessed plasma sample at room temperature for 4 h	20.2	1,628	4.23	345
RE %	0.92	1.77	5.75	7.92
Three freeze/thaw cycles	18.2	1,430	3.72	296
RE %	−9.00	−10.6	−6.92	−7.50
Long-term 44 days (−70°C)	20.1	1,567	4.25	317
RE %	0.67	−2.08	6.17	−0.94

Abbreviation: RE, relative error.

Pharmacokinetic and BE studies

The LC–MS/MS method was employed for determining PK parameters of flupirtine and D-13223 in healthy male Chinese volunteers after administration of test and reference capsules at a dose of 100 mg. The mean plasma concentration–time profiles of flupirtine and D-13223 under fasting condition are depicted in Figure 2A, and the mean plasma concentration–time profiles of flupirtine and D-13223 under fed condition are depicted in Figure 2B. The main PK parameters of flupirtine and D-13223 under fasting condition are summarized in Table 2 and under fed condition in Table 3. The results of ANOVA test of two formulations for flupirtine and D-13223 under fasting and fed conditions are summarized in Table 4. The geometric mean ratios (90% CI) of the test/reference capsules for flupirtine and D-13223 are summarized in Table 5.

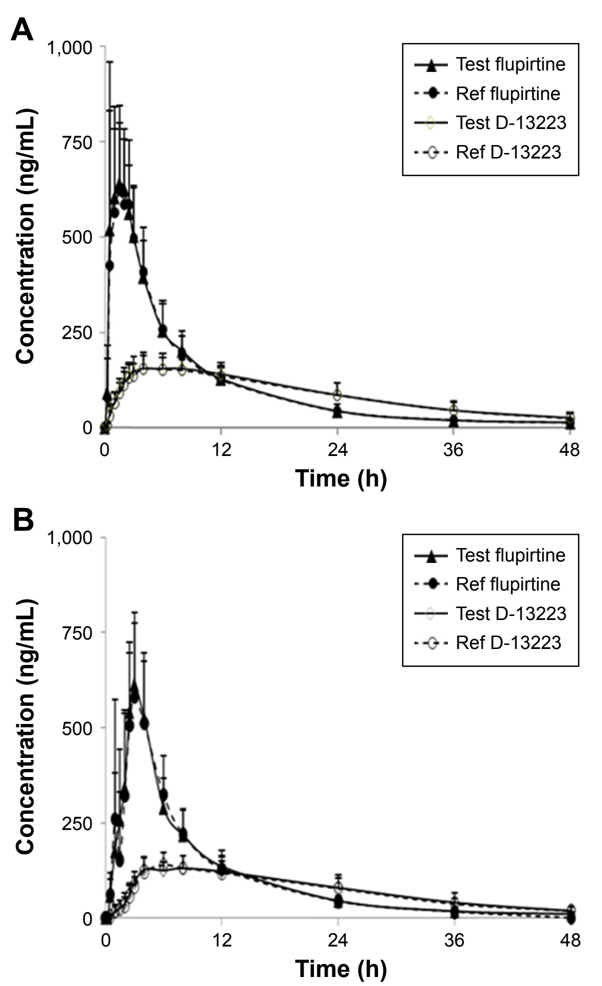

Figure 2 Mean plasma concentration–time profiles of flupirtine and D-13223 under fasting (**A**) and fed (**B**) conditions after an oral dose of 100 mg test capsule and reference capsule.
Note: Each profile represents the mean + SD of 24 volunteers.

Table 2 Pharmacokinetic parameters for two formulations under fasting condition

Fasting	Flupirtine		D-13223	
	Reference	Test	Reference	Test
T_{max} (h)	1.50±0.82	1.52±0.73	5.98±3.17	5.54±2.76
C_{max} (μg/mL)	0.83±0.23	0.82±0.27	0.17±0.04	0.18±0.01
AUC_{0-t} (μg × h/mL)	4.99±1.24	5.12±1.18	4.08±1.13	4.16±1.06
$AUC_{0-\infty}$ (μg × h/mL)	5.21±1.25	5.33±1.16	4.60±1.43	4.71±1.41
$t_{1/2}$ (h)	7.64±1.57	7.84±1.59	13.4±3.12	13.4±2.82

Abbreviations: AUC_{0-t}, area from time zero to the last measurable concentration; $AUC_{0-\infty}$, area under the plasma concentration–time curve from administration to infinite time.

Table 3 Pharmacokinetic parameters for two formulations under fed condition

Fed	Flupirtine		D-13223	
	Reference	Test	Reference	Test
T_{max} (h)	3.04±0.78	2.87±0.64	7.04±2.82	6.30±2.29
C_{max} (μg/mL)	0.65±0.21	0.67±0.18	0.15±0.03	0.15±0.04
AUC_{0-t} (μg × h/mL)	4.63±1.14	4.76±1.14	3.43±0.93	3.50±1.16
$AUC_{0-\infty}$ (μg × h/mL)	4.83±1.12	4.96±1.13	3.78±1.20	3.83±1.38
$t_{1/2}$ (h)	7.54±1.19	7.72±1.29	12.0±2.18	11.4±2.20

Abbreviations: AUC_{0-t}, area from time zero to the last measurable concentration; $AUC_{0-\infty}$, area under the plasma concentration–time curve from administration to infinite time.

The results show that under both fasting and fed conditions, there were no difference in interindividuals, interpreparations, and interperiods between test and reference capsules. The 90% CI of the test/reference AUC ratio and C_{max} ratio of flupirtine and D-13223 were within the acceptance range for BE.

Safety

A total of 104 volunteers were screened and 48 volunteers were enrolled. No volunteer enrolled withdrew from the study, and both the formulations were well tolerated. No severe or unexpected adverse event (AE) was recorded except the laboratory examination, which returned to normal after 8 days without the use of any concomitant medication.

Discussion

LC–MS/MS method development and optimization

Various methods are available for the determination of flupirtine alone or flupirtine along with its major active metabolite D-13223 in biological fluids, like determination of flupirtine and D-13223 by LC–MS/MS following deproteinization with cyanomethane in rat,[18] liquid–liquid extraction with

Table 4 Results of ANOVA test of two preparations under fasting and fed conditions

Parameters	Flupirtine			D-13223		
	Interindividuals	Interpreparations	Interperiods	Interindividuals	Interpreparations	Interperiods
Fasting						
Ln (C_{max})	0.56	0.67	0.71	0.18	0.08	0.11
Ln (AUC_{0-t})	0.80	0.31	0.96	0.60	0.40	0.32
Ln ($AUC_{0-\infty}$)	0.83	0.31	0.98	0.71	0.32	0.23
Fed						
Ln (C_{max})	0.39	0.25	0.99	0.94	0.64	0.10
Ln (AUC_{0-t})	0.87	0.14	0.15	0.88	0.71	0.15
Ln ($AUC_{0-\infty}$)	0.82	0.12	0.22	0.88	0.92	0.66

Abbreviations: ANOVA, analysis of variance; AUC_{0-t}, area from time zero to the last measurable concentration; $AUC_{0-\infty}$, area under the plasma concentration–time curve from administration to infinite time.

Table 5 Statistical comparison of the two formulations

Dependent	Flupirtine				D-13223			
	Ratio_% ref	CI_90 lower	CI_90 upper	Power	Ratio_% ref	CI_90 lower	CI_90 upper	Power
Fasting								
Ln (C_{max})	97.0	85.9	109.5	0.92	105.5	101.5	109.7	1.00
Ln (AUC_{0-t})	103.0	98.1	108.2	1.00	102.4	98.7	106.2	1.00
Ln ($AUC_{0-\infty}$)	102.9	98.2	107.9	1.00	102.6	99.2	106.1	1.00
Fed								
Ln (C_{max})	103.5	94.7	113.0	0.99	98.0	91.9	104.4	1.00
Ln (AUC_{0-t})	101.7	98.4	105.1	1.00	100.6	97.2	104.3	1.00
Ln ($AUC_{0-\infty}$)	101.6	98.5	104.8	1.00	102.6	99.2	106.1	1.00

Abbreviations: AUC_{0-t}, area from time zero to the last measurable concentration; $AUC_{0-\infty}$, area under the plasma concentration–time curve from administration to infinite time.

diethyl ether-dichloromethane in humans,[15] and methyl-tert-butylether in humans.[19,20] Also, there are some investigations about flupirtine by high-performance liquid chromatography coupled with fluorescence derivatization (HPLC–FLD) in animals or humans.[21–25] Based on literature survey, we developed an LC–MS/MS method for simultaneous quantification of flupirtine and D-13223 in human plasma, which compared with previous studies,[15,19,20] was optimized in the plasma volume and sample processing without controlling the factors like pH and temperature. It also has an adequate linearity range and lower limit of quantitation compared with Kandasamy et al.[18] Also compared to HPLC–FLD, like previous studies,[21–25] the LC–MS/MS method has the advantage of high stabilization and is also time-saving. The LC–MS/MS method used for simultaneous quantification of flupirtine and D-13223 provided the sensitivity, specificity, and high sample throughput required for PK and BE studies.

BE studies

In the present study, the 100 mg katadolon (AWD pharma GmbH Co.KG, LTD, Hannover, Germany) was used as the reference product, not only because of its same active substance, strength, and route of administration to the test product but also because it was the first approved flupirtine preparation in the world and it has the complete and sufficient safety and validity data as the basis for the approved listing.

Healthy volunteers were selected under the eligibility criteria, which were set to ensure that only a subject population without accompanying diseases interfering with the conduct and scientific evaluation of the study were enrolled in the study. In addition, involving healthy volunteers alone would minimize risk to the volunteers' well-being. To perform the two one-sided test procedures for BE on log-transformed plasma flupirtine concentration data, with BE limits of 0.80 and 1.25 for AUC and C_{max}, alpha =0.05, and power =80%, the number of volunteers needed for the BE study was determined by means of CI, as formerly presented by Ou-Yang et al.[23] In the present study, the intrasubject coefficient of variance obtained from the ANOVA for the flupirtine and D-13223 are listed in Table 6. Hence, the number of volunteers in both fasting and fed studies (24 volunteers) ensured that this study had adequate power to confirm the statistical conclusions.

The selection of parent drug and active metabolite as the moieties to be measured for BE assessment fulfilled the US Food and Drug Administration requirements of primary metabolite(s), formed directly from the parent compound. The parent drug and active metabolite should be measured if they are both 1) formed substantially through presystemic metabolism (first-pass, gut wall, or gut lumen metabolism) and 2) contribute significantly to the safety and efficacy of the product. The results of metabolite data provided supportive evidence and further ensured the BE for generic and reference drugs.

Based on standard BE guidelines,[26,27] the criterion for BE is the 90% CI of the test/reference geometric means ratio that falls in the range of 80.00%–125.00% for both AUC and C_{max}. The results of the present study showed that the 90% CI of the test/reference ratios for AUC_{0-t}, $AUC_{0-\infty}$, and C_{max} of flupirtine were within the acceptance range for BE both under fasting and fed conditions.

Using Wilcoxon matched-pairs test on the original data, the difference between the T_{max} values of the two (test and reference) products was not statistically significant. Also the D-13223 data further prove the equivalent conclusion.

Compared with the parameters in Ou-Yang et al[23] and Guo et al,[25] in our study the C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ of flupirtine under fasting condition for the test drug were lower, but T_{max} and $t_{1/2}$ were in alignment with them. The difference of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ between our study and Ou-Yang et al[23] and Guo et al[25] may have been caused by the different detector. However, the T_{max} and $t_{1/2}$ in all these studies showed the same trend of flupirtine in healthy male Chinese. The PK parameters are summarized in Table 7.

Food effects on bioavailability of flupirtine

So far, no reports of PK of flupirtine under fed condition in Chinese have been found in China or abroad. In this study, the PK parameters of flupirtine and D-13223 were compared between fasting and fed conditions (data shown in Tables 2 and 3).

Table 6 The intrasubject coefficient of variance of flupirtine and D-13223

Intrasubject (%)	Flupirtine			D-13223		
	Ln (C_{max})	Ln (AUC_{0-t})	Ln ($AUC_{0-\infty}$)	Ln (C_{max})	Ln (AUC_{0-t})	Ln ($AUC_{0-\infty}$)
Fasting	24.8	9.91	9.58	10.2	9.61	8.79
Fed	18.0	6.67	6.23	12.5	7.26	7.46

Abbreviations: AUC_{0-t}, area from time zero to the last measurable concentration; $AUC_{0-\infty}$, area under the plasma concentration–time curve from administration to infinite time.

Table 7 Summary of flupirtine PK parameters in literatures

PK parameters	Reference literature	Method	C_{max} (μg/mL)	T_{max} (h)	AUC_{0-t} (μg × h/mL)	$AUC_{0-\infty}$ (μg × h/mL)	$t_{1/2}$ (h)
Test capsule	Our study (n=24)	LC–MS/MS	0.82±0.27	1.52±0.73	5.12±1.18	5.33±1.16	7.84±1.59
Katadolon	Our study (n=24)	LC–MS/MS	0.83±0.23	1.50±0.82	4.99±1.24	5.21±1.25	7.64±1.57
	23 (n=24)	LC–FLD	1.25±0.29	2.0±1.2	9.11±2.31	9.62±2.29	8.0±1.1
	25 (n=10)	LC–FLD	1.08±0.30	1.7±0.8	8.41±1.62	8.97±2.00	8.85±2.15

Abbreviations: AUC_{0-t}, area from time zero to the last measurable concentration; $AUC_{0-\infty}$, area under the plasma concentration–time curve from administration to infinite time; LC–FLD, liquid chromatography coupled with fluorescence derivatization; LC–MS/MS, high performance liquid chromatography coupled with a tandem mass spectrometry detector; PK, pharmacokinetic.

The C_{max} of fasting and fed conditions were 0.83±0.23 and 0.65±0.21 μg/mL, respectively. The T_{max} were 1.50±0.82 and 3.04±0.78 h, respectively. It is not difficult to find that the high-fat diet affects the absorption rate of flupirtine, and the T_{max} is prolonged, and the C_{max} is reduced. The AUC_{0-t} of fasting and fed conditions were 4.99±1.24 and 4.63±1.14 μg × h/mL, $AUC_{0-\infty}$ were 5.21±1.25 and 4.83±1.12 μg × h/mL, respectively. The AUC_{0-t} and $AUC_{0-\infty}$ shows a little lower in absorption extent of flupirtine after meal. The results suggest that it is reasonable to take flupirtine in an empty stomach because of its faster absorption rate and complete absorption, which mean faster and better drug efficacy.

Conclusion

The study developed an LC–MS/MS method to simultaneously determine plasma concentrations of flupirtine and its major active metabolite D-13223, and the method was successfully applied to evaluate the BE of test flupirtine maleate capsules and katadolon among healthy male Chinese volunteers under fasting and fed conditions.

Based on the pharmacokinetic and statistical results of the study, we can conclude that the two formulations of flupirtine maleate capsule were bioequivalent (the test and reference product) under fasting and fed conditions, and thus both can be used interchangeably in the clinical setting.

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Disclosure

The authors report no conflicts of interests in this work.

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