The effects of sodium ferulate on the pharmacokinetics of bupropion and its active metabolite in healthy men

L.-C. GAO[1,2], X. HUANG[2], Z.-R. TAN[1], L. FAN[1,2], H.-H. ZHOU[1]

[1]Pharmacogenetics Research Institute, Institute of Clinical Pharmacology, Hunan Key Laboratory of Pharmacogenetics, Central South University, Changsha, Hunan, China
[2]TCM Pharmacogenetics Laboratory, Central South University, Changsha, China

Abstract. – AIMS: Sodium ferulate (SF) is widely prescribed in clinic for the treatments of cardiovascular and cerebrovascular diseases. In our study, we investigated the effect of SF on the pharmacokinetics of bupropion and its active metabolite 4-hydroxybupropion in healthy Chinese men.

METHODS: In a two-phase, randomized, crossover study with a 2-week wash-out period between phases, nineteen healthy male volunteers were given with or without pretreatment with SF 150 mg three times daily for fourteen days, and a single dose of bupropion 150 mg was administered. Pharmacokinetics profiles for bupropion and 4-hydroxybupropion were determined.

RESULTS: After SF treatment relative to bupropion alone, the geometric mean ratios and 90% confidence interval of 4-hydroxybupropion were 1.25 (1.17, 1.34) for $AUC_{0-\infty}$, 1.10 (1.05, 1.15) for C_{max}, and 1.14 (1.09, 1.20) for $t_{1/2}$ respectively. The corresponding values for bupropion were 0.907 (0.811, 1.014), 0.974 (0.910, 1.045) and 1.13 (1.06, 1.21) respectively. The corresponding values for $AUC_{0-\infty}$ ratio of 4-hydroxybupropion over bupropion were 1.28 (0.811, 1.014).

CONCLUSIONS, Bupropion hydroxylation was slightly induced by the 14 days of SF pre-treatment. Whether co-administration with SF require a dosage adjustment of bupropion needs further exploration.

Key Words:
   Sodium ferulate, Bupropion, 4-hydroxybupropion, Pharmacokinetics.

Introduction

Sodium ferulate (3-methoxy-4-hydroxy-cinnamate sodium, $C_{10}H_9NaO_4$, SF; Figure 1), the sodium salt of ferulic acid, is an effective component of Chinese herbal medicines including *Angelica sinensis*, *Cimicifuga heracleifolia* and *Lignsticum chuangxiong*. With the beneficial pharmacological effects such as antioxidant, anti-inflammatory, anti-platelet aggregation, anti-thrombosis, anti-peroxidation, SF has been approved by State Food and Drugs Administration of China (SFDA) as a clinical therapy of cardiovascular and cerebrovascular diseases[1-4]. It always occurs that SF is used combined with other drugs in clinic due to the wide clinical applications of SF. However, the effect of SF on the pharmacokinetics of other coadministrated drugs has seldom reported.

Bupropion (INN, amfebutamone) is a widely used antidepressant and an aid to tobacco cessation. Drug interactions involving bupropion and potential CYP2B6 inhibitors or inducers have been extensively studied[5-12]. Previous study has found that SF could inhibit CYP450 enzyme activities in mice[13].

Till now, no published studies have investigated drug interactions involving SF and bupropion. Thus, the present study was conducted to assess the effect of SF on the pharmacokinetics of bupropion and its major active metabolite 4-hydroxybupropion in healthy volunteers. Because the disposition of bupropion, one of the CYP2B6 substrates, is affected by the genetic polymorphisms of *CYP2B6*, commonly accepted *CYP2B6* alleles consisting of a combination of the nucleotides at cDNA positions 516 and 785 were assessed in our study.

Methods

Subjects

The study protocol was approved by the Ethics Committee of Central South University, Changsha, Hunan, P. R. China (Approved Number: CTXY-110003) and registered in the Chinese Clinical Trial Registry (registration number: ChiCTR-TRC-10001285). Nineteen healthy,
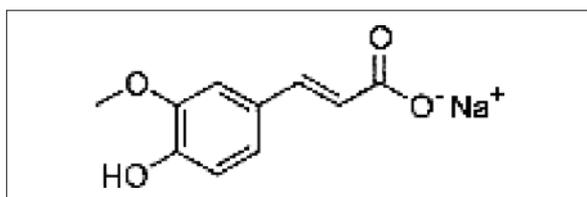
Figure 1. Chemical structures of sodium ferulate.

non-smoking male volunteers of CYP2B6 wild type were enrolled in the clinical trial after having given written informed consent (aged 20 to 34 years; weight range, 53-79 kg; body mass index range, 19-26 kg/m^2). The subjects were ascertained to be in good health by medical history, a full clinical examination, and standard hematologic and blood chemical laboratory tests, and written informed consent for all the participation in the study was obtained before enrollment. Standardized protein rich diets with no vegetables, fruit item or cereal were provided for all the subjects for 2 weeks prior to study and during the whole study to exclude the influence of food-originated SF. Drugs, alcohol, soft drinks, smoking and caffeine-containing beverages, any vitamins and nutritional supplements were refrained for 2 weeks before study commencement and throughout the study.

Study Design

This study was carried out in a two-phase, randomized, crossover manner with a 2-week washout period between phases. In each phase, after an overnight fast, subjects were given pretreatment with or without three 50-mg SF tablets of the same batch (Lot No.: 100810; HengDa ShengKang Pharmaceutical Co., SiChuan, China) for fourteen days. On the fifteenth day, they ingested a single oral dose of 150 mg bupropion (two tablets of 75 mg Zyban SR; WanTe, Hainan, China) with 200 mL water. Blood samples for pharmacokinetic analysis were taken for 72 hours. The subjects did not consume any fluids for 2 hours and food for 4 hours after each dose of bupropion. Standard meals and food were provided for all of the participants. Frequent telephone reminders and detection of plasma concentrations of ferulic acid were carried out to assure subject compliance to treatment. Serial blood samples (5 ml) were collected from an in-dwelling venous catheter (anticoagulated with sodium heparin) before and at 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, 24, 36, 48, 60 and 72 h after bupropion ingestion.

Drug Concentration Analysis

The plasma concentration of bupropion and 4-hydroxybupropion was performed by liquid chromatography-mass spectrometry with using of the Finnigan LCQ Deca XPplus (Finnigan, San Jose, CA, USA). An Angel kromasil C18 (5 μm, 150×4.6 mm) and a mobile phase (acetonitrile: 0.1% ammonium acid: 20 mM ammonium formate = 4:3:3) at a flow rate of 0.2 ml/min were applied. Propranolol was used as the internal standard. The ion transitions monitored were as follows: m/z 240 to 184 for bupropion, m/z 256 to 238 for 4-hydroxybupropion and m/z 260 to 183 for propranolol. These transitions represent the product ions of the [M+H]+ ions. The lower limit of quantification for bupropion and 4-hydroxybupropion were 0.21 ng/ml and 1.172 ng/ml, and the assay range used were 0.42-430.1 ng/ml and 2.344-1200 ng/ml, respectively. Correlation coefficient for bupropion calibration curves were 0.997, and for 4-hydroxybupropion were 0.995. The highest bupropion and 4-hydroxybupropion plasma concentration measured were 323.70 ng/ml and 612.02 ng/ml. The intra-day and inter-day CVs for the low-, middle- and high-quality control samples were less than 10%.

CYP2B6 Genotyping

Genomic deoxyribonucleic acid (DNA) was isolated from peripheral blood samples using SQ Blood DNA Kit (Omega Bio-Tec, Doraville, GA, USA). The wild-type allele *CYP2B6*1* was defined as 516G/785A, and *CYP2B6*6* was defined as 516T/785G. *CYP2B6*6* was detected in a haplotype assay using a two-step allele-specific polymerase chain reaction (PCR) as described previously[14]. The validity of the method was confirmed by sequencing.

Pharmacokinetic Analysis

The maximum plasma concentration (C_{max}) and the time to C_{max} (T_{max}) were obtained by inspection of the concentration-time data. The AUC to the last quantifiable concentration AUC_{0-t} was determined by use of the linear trapezoidal rule. *ke* is the elimination rate constant determined from the terminal slope of the log concentration-time plot. The elimination half-life ($t_{1/2}$) was calculated as $0.693/ke$. The area under the concentration-time curve extrapolated to infinity $AUC_{0-\infty}$ was calculated as $AUC_{0-\infty} = AUC_{0-72} + C72/ke$, where $C72$ is the plasma concentration measured 72 h after drug administration. The

oral clearance (CL/F) of bupropion was calculated by dividing the bupropion dose by the AUC of bupropion and the subject's weight.

Statistical Analysis

Study sample sizes were estimated based on prior bupropion pharmacokinetic data. Geometric mean ratios (GMRs) with 90% confidence intervals (CIs) were calculated after log transformation of within-subject ratios for pharmacokinetic variables for bupropion and 4-hydroxybupropion. Paired two-tailed t-tests were used to test for difference between bupropion alone and in combination with SF, and logarithmic transformation was used for the non-normally distributed data before analysis. The between-treatment t_{max} was compared by use of the Wilcoxon signed rank test. Results are expressed as mean±standard deviation in the text and tables and as mean±standard error in the figures, except for t_{max} data, which are presented as median and range. Statistical calculations were performed with SPSS for Windows, version 11.5 (SPSS Inc., Chicago, IL, USA), and p-values < 0.05 were considered significant.

Results

Nineteen volunteers with CYP2B6*1/*1 were included in and completed the study. No adverse effects were observed in all the subjects.

As shown in Figure 2 and Table I, SF treatment significantly increased C_{max} of 4-hydroxybupropion by 11% (90% CI, 5-15%), $AUC_{0-\infty}$ of 4-hydroxybupropion by 25% (90% CI, 17-34%), and $t_{1/2}$ of 4-hydroxybupropion and bupropion by 14% (90% CI, 9-20%) and 13% (90% CI, 6-21%) respectively. The AUC ratio of 4-hydroxybupropion to bupropion (a measure of CYP2B6 activity) was 14.2 (95% CI, 11.8-16.9) when bupropion was administered alone, and 18.1 (95% CI, 15.5-21.2) when given concurrently with SF. SF elevated the AUC ratio of 4-hydroxybupropion over bupropion by 28% (90% CI, 16-41%; $p < 0.01$). Neither C_{max}, $AUC_{0-\infty}$ and CL/F of bupropion were significantly altered by SF administration. t_{max} values for both bupropion and 4-hydroxybupropion were not changed before and after SF consumption.

Intra-subject changes in the $AUC_{0-\infty}$ for 4-hydroxybupropion, bupropion and AUC ratio of 4-hydroxybupropion over bupropion are depicted in Figure 3(A), (B) and (C), respectively. It can be seen in Figure 3 (A) and (C) that only one subject had lower plasma exposure to 4-hydroxybupropion and lower AUC ratio of 4-hydroxybupropion over bupropion after administration with SF.

Discussion

In current investigation, confounding factors such as age, sex, smoking habit and diet are unified for better interpretation of the results.

SF is the sodium form of ferulic acid, a natural component of traditional Chinese herbs such as *Angelica sinensis* and *Cimicifuga heracleifolia* and some foodstuffs (e.g. grain and some fruits). SF has a variety of pharmacological activities for immunity system, ischemia-reperfusion injury, kidney and liver damage, chronic complications of diabetes and so on, and could be used combined with many drugs in clinic. A comparatively long-term usage might add risk for potential drug interactions.

The efficacy and safety of bupropion have been indicated to be related to plasma concentrations of bupropion and the active metabolite 4-
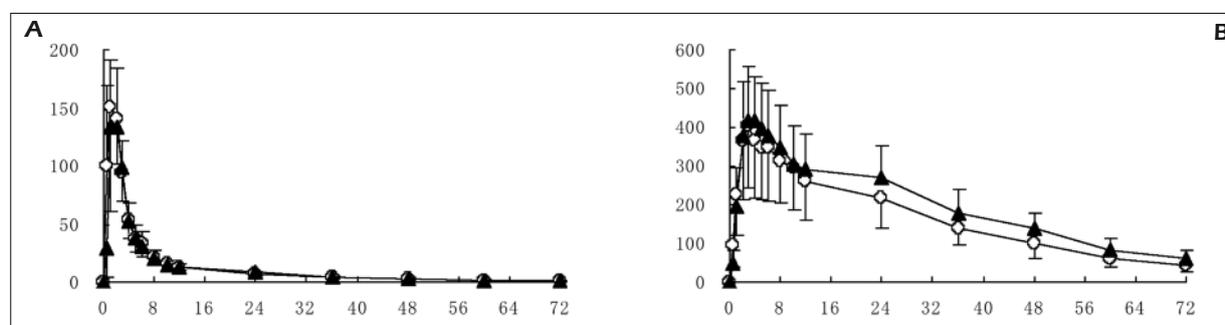

Figure 2. Plasma concentration (conc)-time profiles of bupropion *(A)* and 4-hydroxybupropion *(B)* after single oral dose alone (open circles) or during treatment with sodium ferulate (solid triangles). Data for nineteen healthy subjects are presented as mean ± SE.

Table I. Pharmacokinetic parameters (mean±SD) of bupropion and 4-hydroxybupropion after single oral dose of 150 mg bupropion alone or co-administered with daily sodium ferulate in healthy subjects.

Parameter	Bupropion alone (n = 19)	Bupropion and SF administration (n = 19)	Geometric. mean ratios (90% CI)	p
Bupropion				
$AUC_{(0-\infty)}$ (ng·h/ml)	904.1 ± 240.1	877.0 ± 191.1	0.974 (0.910, 1.045)	0.599
C_{max} (ng/mL)	174.4 ± 44.0	161.4 ± 53.1	0.907 (0.811, 1.014)	0.146
t_{max} (h) (h)a	1.0 (0.5-2)*	1.5 (1-3)*		0.055
$t_{1/2}$ (h)	10.2 ± 2.2	11.4 ± 1.7	1.13 (1.06, 1.21)	0.005
CL/F (L h^{-1} kg^{-1})	2.69 ± 0.54	2.79 ± 0.70	1.03 (0.96, 1.10)	0.506
4-hydroxybupropion				
$AUC_{(0-\infty)}$ (ng·h/ml)	13174 ± 4613.6	16330 ± 5360.3	1.25 (1.17, 1.34)	< 0.001
C_{max} (ng/mL)	416.0 ± 167.4	445.7 ± 139.1	1.11 (1.05, 1.15)	0.003
t_{max} (h) (h)a	3 (2-6)*	4 (2-6)*		0.218
$t_{1/2}$ (h)	22.5 ± 2.99	25.8 ± 3.80	1.14 (1.09, 1.20)	< 0.001
Hydroxybupropion $AUC_{0-\infty}$/ bupropion $AUC_{0-\infty}$	15.1 ± 6.0	19.0 ± 6.3	1.28 (0.811, 1.014)	< 0.001

Median (ranges). $p < 0.05$ is indicated.

hydroxybupropion[16,17]. There are three active metabolites for bupropion, in which 4-hydroxybupropion is likely responsible for much of the pharmacologic activity observed with bupropion administration with approximately half of the pharmacologic activity of bupropion and 17-fold higher than AUC of bupropion at steady state[18]. In our study, the use of SF resulted in an average of a 25% increase in the AUC of 4-hydroxybupropion in nineteen healthy male subjects, a longer bupropion half-life without alterations in the exposure of the parent drug. Whether these mild changes would be of any clinical significance awaits for further investigations.

Hydroxybupropion plasma AUC and the AUC ratio of hydroxybupropion to bupropion were used for assessing constitutive CYP2B6 activity[29-32]. In our study, the use of SF resulted in an average of a 28% increase in the AUC ratio of hydroxybupropion over bupropion and a 25% increase in the AUC of hydroxybupropion, without significant change in bupropion clearance. The increase in the AUC ratio during SF co-administration is consistent with an increased proportion of the hydroxybupropion via metabolism catalyzed by CYP2B6, indicating SF induction on CYP2B6-catalyzed bupropion hydroxylation. However, since the elimination half-life of hydroxybupropion was significantly extended, the possibilities of decreased elimination of hydroxybupropion by SF cannot be excluded. Also, since the pathway for 4-hydroxybupropion elimination has not been clarified, and exposure of the other two metabolites of bupropion has not been measured, the potential mechanism involved by SF needs further explorations.
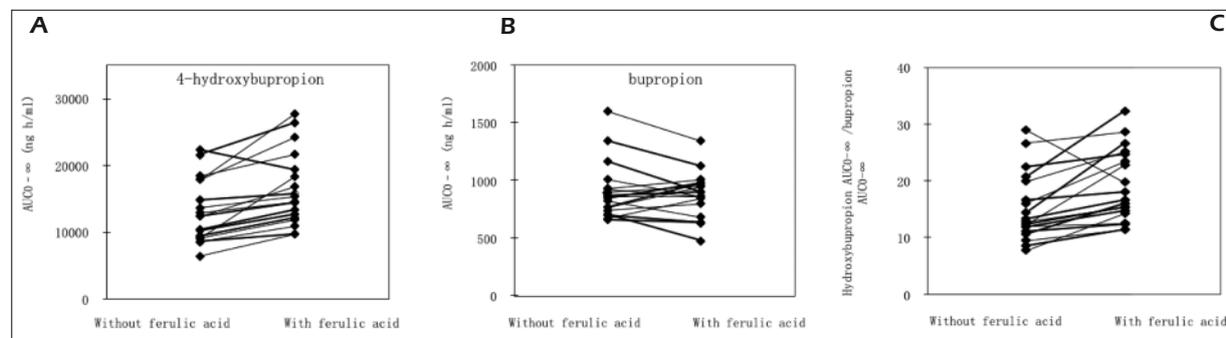
Figure 3. Individual values for 4-hydroxybupropion $AUC_{(0-\infty)}$ *(A)*, bupropion $AUC_{(0-\infty)}$ *(B)* and the AUC ratio of 4-hydroxybupropion to bupropion *(C)* in nineteen healthy subjects after single oral dose of bupropion with and without daily coadministration with sodium ferulate.

In conclusion, this is the first study to investigate the interaction between bupropion and SF. Results of the present study indicate that 14-day of regular dosage of SF has mild effect on the pharmacokinetics of the active metabolite of bupropion, 4-hydroxybupropion without alteration for bupropion clearance. The results will add more information to the use of bupropion in patients with SF.

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