Bioequivalence Evaluation of Two Tablet Formulations of Carbocysteine in Healthy Chinese Men

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ABSTRACT

A randomized, two-way crossover study was conducted in 20 healthy Chinese male volunteers to compare the bioequivalence of two tablet formulations of carbocysteine as required by China State Food and Drug Administration. Test and reference tablets were administered as a single dose on two treatment days separated by a 1-week washout period. After dosing, serial blood samples were collected for a period of 10 hours. Carbocysteine in human plasma was determined by a sensitive, selective, reproducible and accurate liquid chromatography-tandem mass spectrometry (LC/MS/MS) method validated following international guidelines.

Pharmacokinetic parameters including C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, and AUC to infinity ($AUC_{0-\infty}$) were determined from plasma concentration of both formulations, and it is interesting to find that there is maybe ethnic variation in the C_{max} value of carbocysteine. Statistical modules (analysis of variance [ANOVA] and 90% confidence interval [CI]) were applied to C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ to assess the bioequivalence of the two tablets. No significant differences between the tablets were found, and the 90% CI fell within the China and US FDA accepted range of 80% to 125%. The results indicate that the two tablet formulations of carbocysteine are equivalent in the rate and extent of absorption.

INTRODUCTION

Carbocysteine, *S*-carboxymethyl-*L*-cysteine (chemical structure shown in Figure 1), a dibasic amino acid, is a mucoregulating agent. It diminishes the viscosity and increases the volume of pathologically thickened sputum, thereby facilitating expectoration.[1] In clinical

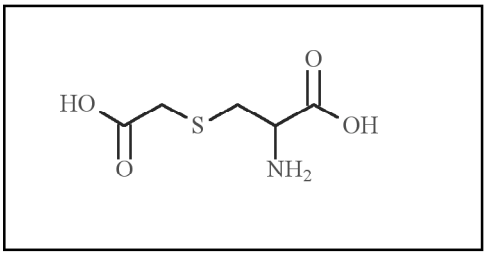

Figure 1. Chemical structure of carbocysteine

practice carbocysteine has gained acceptance and is used in the management of respiratory diseases characterized by accumulation of excessive secretions.[1]

Carbocysteine is administered orally in liquid or solid dosage forms including syrup, tablet and capsule.[2] Carbocysteine is rapidly well absorbed after oral administration and the subsequent kinetics fit a one-compartment open model.[1,3] Peak serum concentrations are reached between 1 and 2.0 hr and peak values were 10.8 to 13.9 mg/L after a 1500 mg dose.[3,4] C_{max} of 8.2 μ/mL at 3.0 hr was observed after administration of 750 mg of carbocysteine in capsule.[5] C_{max} of about 13.0 μg/mL was observed after administration of 1000 mg of carbocysteine in granule or suspension.[6] The plasma half-life was estimated to be 1.33 hr and the apparent volume of distribution was approximately 60 L. There is no information on intravenous studies to allow bioavailability determination.[1] There is no reported work on first-pass metabolism or protein binding.[1]

Carbocysteine appears to penetrate well into lung tissue[7] and respiratory mucus,[8] suggesting local action. There are no data to suggest a relevant relationship between concentration and effect.[1] Significant variation between the patterns of metabolism in humans and animals has been noted.[9,10] There are no reports of pharmacologically important activity in these metabolites. The majority of the drug is eliminated unchanged by urinary excretion.[9,10.]

Recently a new dispersible tablet formulation of carbocysteine has been developed by Bai-yun-shan Pharm (Guangzhou, China). Bioequivalence study between this new formulation and the first approved tablet formulation (also manufactured by Bai-yun-shan Pharm.) in China is required by the State Food and Drug Administration in order to register and market this new formulation in China. The aim of the present work was to determine bioequivalence between these two products containing carbocysteine and to ascertain equal effect and safety in medical practice in our population.

MATERIAL AND METHODS

Study Products

The test formulation was carbocysteine 100 mg dispersible tablet (Batch No. 040416, Expiry date: 03/06, from Bai-yun-shan Pharm). The reference product was carbocysteine 250 mg tablet (Batch No. 0404011, Expiry date: 03/06, also from Bai-yun-shan Pharm). The reference number of clinical trial of carbocysteine approved by State Food and Drug Administration was 2004L01252.

Study Subjects

Twenty healthy adult male volunteers completed this study at the First Affiliated Hospital, Sun Yat-sen University, China. Their mean age was 23.0 ± 1.5 years with a range of 20 to 26 years. Mean height was 169.0 ± 4.9 cm with a range of 160 to 178 cm and mean body weight was 60.3 ± 5.2 kg with a range of 52 to 70 kg. The volunteer subjects were selected after completing a thorough medical history and physical examination, and after a normal laboratory examination (hematology, blood biochemistry, and urine analysis). The volunteers had no evidence of hepatic, renal, pulmonary, cardiac, gastrointestinal, neurologic, or hematologic disorders or any acute or chronic disease. None of

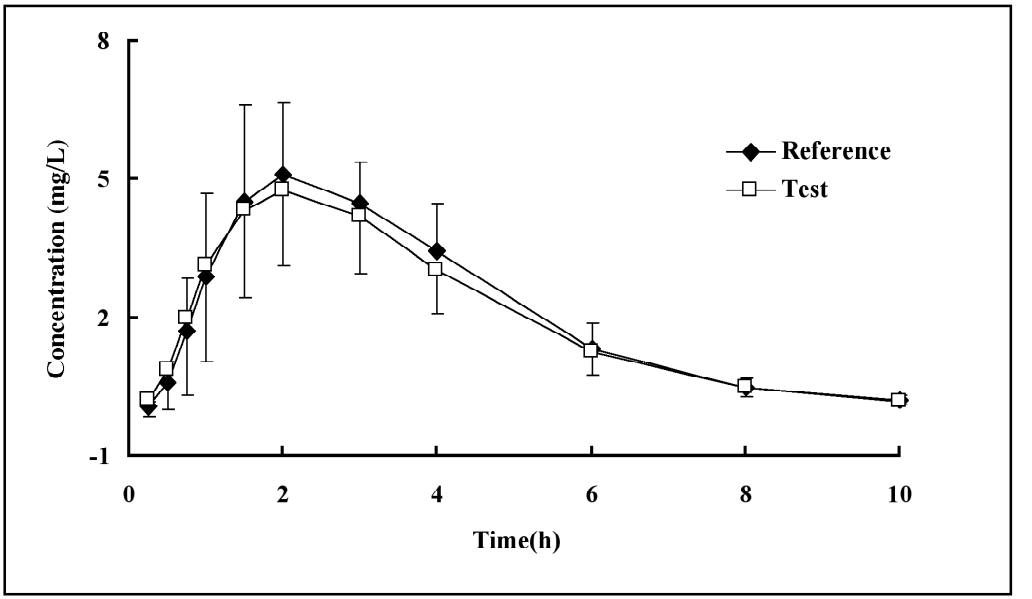

Figure 2. Mean plasma concentration of carbocysteine after oral administration of single dose of 1000 mg of two tablet formulations to 20 healthy male volunteers.

the subjects smoked. Subjects confirmed that they had abstained from taking alcohol, caffeine, or caffeine-containing beverages or food for 48 hr prior to the study and from the time of drug administration until the last blood sample was collected. Subjects were instructed to abstain from taking any drug, including over-the-counter (OTC) products, for at least 2 weeks prior to and during the study period. Written informed consent was obtained from the subjects after explaining the aim and risks of the study. The study was performed according to the revised Declaration of Helsinki for biomedical research involving human subjects and the rules of current Good Clinical Practices. The study protocol was approved by the Human Investigation Ethical Committee of School of Pharmaceutical Sciences at the Sun Yat-sen University, Guangzhou, China.

Drug Administration and Blood Samples Collection

The study was of a single dose, randomized, two treatments, two-period crossover design. In the morning of phase I, after an overnight fast (12 hr), volunteers orally administered a single dose of a 1000-mg carbocysteine tablet with 200 mL of water. Regular standardized low-fat meals were provided until 4 hr after dose administration; water intake was allowed after 2 hr. Water, lunch, and dinner were given to all volunteers according to a time schedule. They were under continual medical supervision at the study site. For carbocysteine analysis, venous blood samples of approximately 3 mL were drawn into heparinized glass tubes through an indwelling cannula at the following times: immediately before administration (0 hr) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, and 10 hr after dosing. Blood samples were centrifuged at 4000 rpm for 10 min, and plasma was transferred into 5 mL glass tubes. The plasma samples were labeled and kept frozen at –30°C until analysis. After a washout period of 7 days the study was repeated in the same manner to complete the crossover design.

Table 1. Pharmacokinetic parameters of two tablet formulations of carbocysteine (mean ± standard deviation, *n*=20)

Parameter	Carbocysteine	
	Reference formulation	Test formulation
C_{max} (μg/mL)	5.8 ± 1.3	5.6 ± 1.4
T_{max} (hr)	2.3 ± 0.9	2.2 ± 0.8
$t_{1/2}$ (hr)	1.5 ± 0.2	1.5 ± 0.1
AUC_{0-t} (μg hr/mL)	21.4 ± 3.9	20.1 ± 4.3
$AUC_{0-\infty}$ (μg hr/mL)	23.9 ± 4.2	22.8 ± 4.5
AUC_{0-t} / $AUC_{0-\infty}$(%)	89.3 ± 2.4	88.0 ± 4.6

Sample Preparation for LC/MS/MS Injection

To the 200-μL plasma sample in 1.5-mL tube, 400 μL of methanol were added. After vortexing for 10 sec and centrifuging at 15000 rpm for 4 min, 20 μL of the clear supernatant was directly injected onto the liquid chromatography-tandem mass spectrometry (LC/MS/MS) system.

Liquid Chromatographic and Mass Spectrometric Conditions

A sensitive, selective, and accurate LC/MS/MS method was developed and validated before the study for carbocysteine determination in plasma samples. All solvents used were of HPLC grade; while other chemicals and reagents were of analytical grade. Carbocysteine, which was provided by Yichang Sanxia Pharmaceutical Co. (Wuhan, China), had a relative purity of 100.1% as compared to the standards from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). A Finnigan LC/MS/MS system (San Jose, CA) consisting of a Surveyor MS pump, a Survey autosampler, and a tandem mass spectrometer equipped with electrospray ionization source was used. Finnigan Xcalibur 1.3 and Finnigan Lcquan software were used for data acquisition and processing.

Chromatographic separation was achieved by using a Hypurity C_{18} column (I.D. 2.1 mm □ 50 mm, 5 μm, Thermo Electron Corporation, USA) at 30°C. The mobile phase consisted of methanol/water (containing 0.1% formic acid) (50:50, v/v), delivered at a flow rate of 200 μL/min. Total run time was 2 min for each injection. Mass spectrometric analysis was performed in the positive ion mode. Detection was done at selected reaction monitoring mode (SRM) of *m*/*z* 180 to 89 for carbocysteine. The peak area was measured and the concentrations were calculated by the Finnigan Lcquan software. The method was validated following international guidelines.[11]

Pharmacokinetic Analysis

Calculation of pharmacokinetic parameters was done by performing UCSF NONMEM version 1.1 software (GloboMax LLC, Hanover, MD) The elimination rate constant (l_z) was obtained as the slope of the linear regression of the log-transformed concentration values versus time data in the terminal phase. The elimination half-life ($t_{1/2}$) was calculated as 0.693/l_z. Time to peak plasma concentration (T_{max}) and peak plasma concentration (C_{max}) were read directly from the observed concentration versus time profiles. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity (AUC_{0}) was calculated as AUC_{0} = AUC_{0-t} + C_t /$_z$, where C_t is the last measurable concentration.

Table 2. Statistical analysis of log-transformed data

Statistical analysis	C_{max}	AUC_{0-t}	$AUC_{0-\infty}$
ANOVA (*P*-value)	0.0579 (0.2399)	0.0682 (0.1167)	0.0863 (0.1638)
90% CI	88.4% - 105.1%	(94.1% - 98.8%)	88.3% - 98.5 %
	(91.3% - 95.3%)	90.7% - 100.9%	(93.1% - 96.4%)

Parenthesis values indicate analysis for periods.
ANOVA= analysis of variance
CI=confidence intervals of the mean test/reference ratio.

Statistical Analysis

For the aim of bioequivalence analysis between two formulations, C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were considered as primary variables. The bioequivalence of the two products was assessed by means of an analysis of variance (ANOVA) for crossover design and calculating standard 90% confidence intervals (CI) of the ratio test/reference (T/R) using log-transformed data. Statistical significance of variations in the different formulations was tested according to an ANOVA test by the Excel 2000 program (Microsoft, Seattle, WA). The products were considered bioequivalent if the difference between the two compared parameters was statistically insignificant ($P > 0.05$) and 90% confidence intervals for these parameters fell within 80% to 125%, which is the range accepted by the US and China State Food and Drug Administration.[12,13]

RESULTS AND DISCUSSION

The described analytical method used for measurement of carbocysteine in plasma was proved to be accurate and sensitive. The lower limit of quantitation was 0.1 µg/mL using 0.2 mL plasma sample. The relationship between concentration and peak area was found to be linear within the range 0.1 to 20.0 µg/mL. The intra-day accuracy of the method for carbocysteine ranged from 95.9% to 100.4%, while the intra-day precision ranged from 3.0% to 4.3%. The inter-day accuracy ranged from 96.4% to 102.7%, while the inter-day precision ranged from 5.1% to 7.0%. The absolute recovery was 87.0% to 89.3% while the relative recovery ranged from 98.86% to 100.4%. A stability study showed that carbocysteine was stable in plasma at room temperature for at least 4 hours, as well as for 23 days at -30°C and after three freeze-thaw cycles.

Formulations used in this study were well tolerated at the dose administered by all the volunteers. Unexpected incidents that could have influenced the outcome of the study did not occur. There were no drop-outs and all volunteers who started the study continued to the end and the biochemical parameters remained unchanged and within the reference range.

Both tablet formulations were readily absorbed from the gastrointestinal tract and carbocysteine was measurable at the first sampling time (0.25 hr) in the majority of the volunteers. The mean concentration-time profiles of two formulations were closely similar and superimposable (Figure 2). ANOVA was applied on the concentration attained at individual time intervals for both formulations, and indicated no significant difference. The peak concentration of the test and reference products was 5.6 µg/mL and 5.8 µg/mL for carbocysteine at 2.2 hr and 2.3 hr after administration, respectively. Concentration then declined but remained detectable up until 10 hr.

The pharmacokinetic parameters for the reference and test formulations are

presented in Table 1. The means and standard deviations of these parameters for the two brands are very similar, indicating that the pharmacokinetics of carbocysteine in the two formulations is also similar. The mean ratio of $AUC_{0\text{-}t}$/$AUC_{0\infty}$ for reference and test formulation of 89.3% and 88.0%, respectively, indicates that the sampling time was adequate.[14] The relative bioavailability of reference formulation was 94.2 ± 13.7% for $AUC_{0\text{-}t}$, 95.6 ± 12.7% for $AUC_{0\infty}$ and 98.7 ± 21.5% for C_{max}.

In the current study, C_{max} values for both formulations ranged from 5.6 to 5.8 μg/mL at 2.2 to 2.3 hr after a single dose of 1000 mg carbocysteine, while Maynard et al reported maximal plasma concentrations of approximately 13.0 μg/mL following administration of 1000 mg of carbocysteine in a suspension or in granules.[6] C_{max} of 13.4 μg/mL at 1.7 hr was observed in a British study after administration of 1500 mg of carbocysteine in capsule.[3] A maximum concentration of 13.9 μg/mL at 2 hr was obtained in Belgian trial after a dose of 1500 mg of carbocysteine powder.[4] C_{max} of 8.2 mg μ/mL at 3.0 hr was observed in German study after administration of 750 mg of carbocysteine in capsule.[5] It can be seen that T_{max} obtained in the current study was in agreement with reported values.[1,3-5] while the C_{max} values in this study was lower than that of the reported values.[1,3-5]

It is interesting to find that there may be ethnic variation in the absorption of carbocysteine. This was not an expected finding. Further studies need to be performed to confirm this interesting finding and to clarify whether it is caused by ethnic variation, ingredients contained in the formulation, or difference in process technique.

The most important objective of any bioequivalence study is to assure the safety and efficacy of the test and reference products. When two formulations of the same drug are equivalent in the rate and extent to which the active drug becomes available to the site of drug action, they are bioequivalent and thus considered therapeutically equivalent.[15] It is generally accepted that equivalent range for basic pharmacokinetic parameters, such as C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\infty}$,is 80% to 125%.[11,12]

The results of our statistical analysis are shown in Table 2. The mean and standard deviation of C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\infty}$ of the two formulations did not differ significantly, suggesting that the plasma profiles generated by the test formulation are comparable to those of the reference formulation. ANOVA, after log-transformation of the data, showed no statistically significant difference between the two formulations (P>0.05). Furthermore, the 90% CI for the ratios of test drug to reference drug for C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\infty}$ were also within the accepted range of 80% to 125%.[11,12] Therefore, the two tablet formulations can be considered bioequivalent with regard to the extent and rate of absorption.

CONCLUSION

Statistical analysis (ANOVA and 90% CI) for C_{max}, $AUC_{0\text{-}t}$, and $AUC_{0\infty}$ clearly indicated no significant difference in the two carbocysteine tablets. Based on the pharmacokinetic and statistical results of this study, it is concluded that Carbocysteine 100 mg Dispersible Tablet is bioequivalent to Carbocysteine 250 mg Tablet (the-first-approved carbocysteine formulation in China), and that the two formulations can be considered equally effect and safe in medical practice.

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