Pharmacokinetics and comparative bioavailability of two oral formulations of tamoxifen citrate in healthy male volunteers

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Abstract  Aim To access the pharmacokinetics and comparative bioavailability of two tamoxifen citrate (TAM) formulations in 20 healthy Chinese male subjects under fasting conditions. Methods TAM test capsules and reference tablets were administered as a single dose on two treatment days separated by a 3-week washout period. After dosing, serial blood samples were collected for a period of 72 hr, and plasma TAM concentrations were determined by a sensitive, selective, reproducible and accurate liquid chromatography-tandem mass spectometry (LC/MS/MS) method. Pharmacokinetic parameters were analyzed by the non-compartmental method. Results The main pharmacokinetic parameters of tamoxifen citrate test and reference formulations were as follow: $T_{\text{max}}$ were $(3.90 \pm 0.91)$ and $(3.95 \pm 1.10)$ h, $C_{\text{max}}$ were $(108.06 \pm 19.84)$ and $(101.26 \pm 16.48)$ µg·L$^{-1}$, $T_{1/2}$ were $(53.52 \pm 14.41)$ and $(51.67 \pm 8.94)$ h, $AUC_{0-72}$ were $(3073.71 \pm 439.08)$ and $(3148.75 \pm 373.70)$ µg·h·L$^{-1}$, $AUC_{0-\infty}$ were $(5074.85 \pm 1082.24)$ and $(5121.18 \pm 902.00)$ µg·h·L$^{-1}$, respectively. The relative bioavailability of test formulations was $(97.88 \pm 10.79)$ %. No significant differences between the two formulations were found, and the parametric confidence intervals (90%) of the mean values of the pharmacokinetic characteristics for test/reference ratio were in each case well within the bioequivalence acceptable ranges of 0.8-1.25 and 0.70-1.43 respectively for $AUC$ and $C_{\text{max}}$. Conclusion The results indicate that the two TAM formulations are equivalent in the rate and extent of absorption.

Key words tamoxifen citrate; LC/MS/MS; pharmacokinetics; bioequivalence

Introduction

Tamoxifen citrate (TAM), 2-[4-(1,2-diphenylbut-1-enyl)phenoxy]-N,N-dimethyl-ethanamine, 2-hydroxypropane-1,2,3-tricarboxylic acid (Fig 1), is an oral selective estrogen receptor modulator (SERM) [1]. TAM is commonly used for adjuvant therapy of breast cancer and is a key drug for chemoprevention of breast cancer in high-risk women[2-4]. It is used for the treatment of early and advanced breast cancer in pre- and post-menopausal women. It is also approved by the Food and Drug Administration (FDA) for the reduction of the incidence of breast cancer in women at high risk of developing the disease. It has been further approved for the reduction of contralateral (in the opposite breast) breast cancer[5,6]. Tamoxifen competes with oestrogen in the body for oestrogen receptors in breast tissue so that transcription of oestrogen-responsive genes is inhibited[7,8].

The purpose of the present work was to determine the pharmacokinetics and bioequivalence
between the two formulations of TAM and to ascertain equal effect and safety in medical practice in our population.

![Chemical structure of TAM](image)

**Fig 1  Chemical structure of TAM**

**Materials and methods**

**Drugs and reagents**

The test formulation was TAM 20 mg capsule provided by Shandong Weifang Pharmaceutical Factory Co., Ltd. (Shandong, China; Batch No. 050222); the reference product was TAM 10 mg tablet manufactured by Shandong Health Pharmaceutical Co., Ltd. (Shandong, China; Batch No. 0604282). The standard substance of TAM was supported from Shandong Health Pharmaceutical Co., Ltd. (purity=99.2%; Batch No. 060302). Loratadine, an internal standard (IS), was supported from Xi’an Xintong Pharmaceutical Co., Ltd. (purity=99.5%, Shanxi, China). Methanol and formic acid were of HPLC grade. Others reagents were of analytical grade. Distilled, deionized water was used in the preparation of all reagents and the mobile phase throughout the study.

**Study subjects**

Twenty healthy adult male volunteers completed this study at Drug Clinical Research Organization of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China). Their mean age was 22.6 ± 1.2 years with a range of 21 to 24 years. Mean height was 168.0 ± 5.4 cm with a range of 160 to 178 cm and mean body weight was 59.4 ± 6.9 kg with a range of 50 to 78 kg. None of the subjects smoked. 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. Subjects confirmed that they had abstained from taking alcohol, cigarette, 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.

The study was conducted in accordance with good clinical practice (GCP) guidelines of State Food and Drug Administration regulation in P.R.China, and the Declaration of Helsinki (as revised in Edinburgh 2000). Approval for the study was gained from the independent Ethical Committee of the Tongji Medical College, Huazhong University of Science and Technology (Wuhan, China) and written informed consent was obtained from all subjects after explaining the aim and risks of the study prior to participation.

**Study design**

The study was of a single dose, randomized, two treatments, two-period crossover design. 20 subjects were randomly divided into 2 groups (test and reference). After an overnight fasting, all subjects were given a single oral dose of a 40 mg TAM test capsules or reference tablets with 250 ml 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. During the whole test period, all subjects remained under closely medical supervision at the study site. A crossover study was followed by a 3-week washout period.

Safety was monitored by routine clinical laboratory tests conducted before and 72 hr after administration of TAM, by recording reported adverse events, and by conducting physical examinations before and after the study.

**Blood samples collection**

Blood samples (5 ml each) were drawn before (0 h) and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 24, 48 and 72 h
after drug administration. An intravenous cannula was placed into the volunteers’ forearm vein before drug administration and left in place until the 72 h blood sample was collected. The blood samples were collected in coded, heparinized centrifuged tubes, immediately subjected to centrifugation at 4000 r·min⁻¹ for 10 min. The plasma was decanted in coded polypropylene tubes and stored at -80 °C until analysis.

Sample preparations for LC/MS/MS analysis

TAM and added internal standard, loratadine, were extracted from 100 µL of human plasma using the methanol. To the 100 µL plasma sample, 1 mL of methanol was added. After vortexing for 10 sec and centrifuging at 10000 r·min⁻¹ for 5 min, the clear supernatant was transferred into another tube. 1 mL of methanol was added into the supernatant, and then the mixture was vortex mixed and centrifuged at 10000 r·min⁻¹ for 5 min. 20 µL of the clear supernatant was injected into the liquid chromatography-tandem mass spectrometry (LC/MS/MS) system as the sample solution.

Liquid chromatographic and mass spectrometric conditions

A Finnigan LC/MS/MS system (Thermo Finnigan, San Jose, CA, USA) equipped with atmospheric pressure chemical ionization (APCI) source and an Agilent 1100 HPLC system (Wilmington, DE) consisting of a vacuum degasser, a binary pump, and an autosampler were used to determine the concentration of TAM in plasma. Finnigan Xcalibur 1.4 software was used for data acquisition and processing. Chromatographic separation was achieved by using a Zorbax SB-C₁₈ column (150 mm×4.6 mm; 5 µm, Agilent, USA) at 25°C. The mobile phase consisted of methanol-water-formic acid (80:20:0.2, v/v/v), delivered at a flow rate of 0.7 mL·min⁻¹. Mass spectrometric analysis was performed in the positive ion mode. All assays were performed by the Center for Drug Metabolism and Pharmacokinetics Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences (Shanghai, China). The lower limit of quantitation was 0.100 µg·L⁻¹ of plasma, and the analytical method demonstrated goodness of fit to an equation \( r=0.9989 \) at plasma TAM concentrations ranging from 0.100 to 1000 µg·L⁻¹. Coefficients of variation for within- and between-batch analyses ranged from 3.5% to 6.3% and 1.3% to 4.6%, respectively.

Pharmacokinetic analysis

Values for peak plasma concentration \( (C_{\text{max}}) \) and time to \( C_{\text{max}} (T_{\text{max}}) \) were taken directly from the observed concentration-time profiles. The terminal-phase half-life \( (T_{1/2}) \) was calculated as \( (\ln2) / K_e \), where \( K_e \) is the slope of the log-linear regression of the terminal concentration data points. The area under the plasma concentration versus time curve from 0 to the last measurable concentration \( (AUC_{0\rightarrow t}) \) was calculated with the linear trapezoidal rule. The area under the plasma concentration versus time curve from 0 to infinity \( (AUC_{0\rightarrow\infty}) \) was calculated as \( AUC_{0\rightarrow t} + C_t / K_e \), where \( C_t \) is the last measurable concentration, and the bioavailability (F) was calculated according to the equation: \( F=\frac{AUC_{0\rightarrow t(\text{test})}}{AUC_{0\rightarrow t(\text{reference})}}<100\% \). Systemic oral clearance (CL) was calculated as dose\( /AUC_{0\rightarrow\infty} \). The pharmacokinetic parameters were calculated by Drug and Statistics Software (DAS, ver2.0) (Mathematical Pharmacology Professional Committee of China).

Statistical analysis

For the aim of bioequivalence analysis between two formulations, \( C_{\text{max}}, AUC_{0\rightarrow72}, \) and \( AUC_{0\rightarrow\infty} \) 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. The parameter \( T_{\text{max}} \) was analyzed with Wilcoxon’s rank sum test. In addition, bioequivalence between the two formulations was evaluated by paired two-one-sided \( t \)-test. 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 0.8-1.25 and 0.70-1.43 respectively for \( AUC \) and \( C_{\text{max}} \), which is the range accepted by the US and China State Food and Drug Administration\(^{[9-11]} \). All data were expressed as mean ± SD. A two-tailed P value of <0.05 was considered statistically significant.
Results

Pharmacokinetics and bioavailability

The mean plasma concentration-time curves after oral administration of TAM formulations in 20 healthy volunteers are shown in Fig 2, and corresponding main pharmacokinetic parameters were listed in Table 1.

The statistic analysis showed that there were no significant differences for pharmacokinetic parameters $AUC_{0-72}$, $AUC_{0-\infty}$, $C_{\text{max}}$, $T_{\text{max}}$ and $T_{1/2}$ between the two formulations ($P>0.05$). The extent of absorption is a key characteristic of drug formulation and, therefore AUC is an important parameter for comparative bioequivalence study. However, the other two parameters, $C_{\text{max}}$ and $T_{\text{max}}$, are also important features and could affect the therapeutic behavior of a drug and hence were also considered in the study. The relative bioavailability for test formulation was $(97.88 \pm 10.79)\%$. The 90% confidential interval of $AUC_{0-72}$, $AUC_{0-\infty}$, $C_{\text{max}}$, of test formulations were 93.5%~101.4%, 91.9%~105.4% and 100.5%~112.5%, respectively. According to the bioequivalence criteria, the two formulations were bioequivalent.

![Mean plasma concentration-time curves of TAM after a single oral administration of 40 mg TAM test and reference formulations in healthy volunteers. $n=20$. Mean $\pm$ SD](image)

Table 1. Main pharmacokinetic parameters of TAM after a single oral dose of 40 mg TAM in healthy volunteers. $n=20$. Mean $\pm$ SD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Test formulations</th>
<th>Reference formulations</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$/µg·L$^{-1}$</td>
<td>108.06±19.84</td>
<td>101.26±16.48</td>
</tr>
<tr>
<td>$T_{\text{max}}$/h</td>
<td>3.90±0.91</td>
<td>3.95±1.10</td>
</tr>
<tr>
<td>$T_{1/2}$/h</td>
<td>53.52±14.41</td>
<td>51.67±8.94</td>
</tr>
<tr>
<td>$AUC_{0-72}$/mg·h·L$^{-1}$</td>
<td>3073.71±439.08</td>
<td>3148.75±373.70</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$/mg·h·L$^{-1}$</td>
<td>5074.85±1082.24</td>
<td>5121.18±902.00</td>
</tr>
<tr>
<td>$F_{0-72}%$</td>
<td>97.88±10.79</td>
<td></td>
</tr>
<tr>
<td>$F_{0-\infty}%$</td>
<td>99.83±17.09</td>
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</tbody>
</table>

Safety evaluation

Both formulations used in this study were well...
tolerated at the dose administered by all the volunteers. Unexpected adverse events that could have influenced the outcome of the study did not occur. None of the changes in laboratory test values and vital signs during the study were considered clinically important. 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.

Discussion

A single oral dose of 40 mg TAM test capsules and reference tablets were given to 20 healthy male Chinese volunteers in a single dose, randomized, two-treatment, two-period crossover study, and its concentrations in plasma were determined with the validated LC/MS/MS. The present pharmacokinetic study indicated that both formulations were readily absorbed from the gastrointestinal tract with a T_max of approximately 3.9 h. The mean concentration-time profiles of two formulations were closely similar and superimposable (Fig 2). The peak concentration of the test and reference products was 108.06 µg·L⁻¹ and 101.26 µg·L⁻¹ for TAM, respectively. Concentration then declined and remained detectable up until 72 hr after administration. The pharmacokinetic results were almost consistent with other reports.[12,13] Throughout the whole study period, there were no adverse events reported.

In our study, the mean and standard deviation of C_max, AUC₀₋₇₂, and AUC₀₋∞ of the two formulations did not differ significantly, suggesting that the plasma profiles generated by the test formulation were 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 parametric confidence intervals (90%) of the mean values of the pharmacokinetic characteristics for T/R ratio were in each case well within the bioequivalence acceptable ranges of 0.8-1.25 and 0.70-1.43 respectively for AUC and C_max. These results were confirmed by the Schuirmann's two one-sided t tests, which indicated that the lower and upper limits of the calculated t value were greater than the critical t value for the three parameters. Therefore, the two TAM formulations can be considered bioequivalent with regard to the extent and rate of absorption.

In conclusion, statistical analysis (ANOVA and 90% CI) for C_max, AUC₀₋₇₂, and AUC₀₋∞ clearly indicated no significant difference in the two TAM formulations. Based on the pharmacokinetic and statistical results of this study, it is concluded that the TAM 20 mg capsule and TAM 10 mg tablet formulations are bioequivalent, and that the two formulations can be considered equally effect and safe in medical practice.

References

12. Tukker JJ, Blankenstein MA, Nortier JW. Comparison of


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