Study on bioequivalence of chlorzoxazone tablets in Chinese volunteers

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Abstract
Aim Chlorzoxazone is analgesic muscle relaxants. On clinic it is used to treat painful muscle spasms. The aim of the study is to investigate human bioequivalence of chlorzoxazone tablets.

Methods Eight healthy male volunteers were recruited. They were divided to two groups randomly. A HPLC was used to determine the blood concentrations of chlorzoxazone. In the morning 400mg of reference drug or test drug was taken orally by the fast volunteers. Blood samples for pharmacokinetic analyses were collected at pre-dose and then at 0.25, 0.5, 1, 1.5, 2, 3, 4 and 6 hours after dosing. One week later, the same dosage of reference or test drug was taken alternatively. A HPLC was used to determine the blood concentrations of chlorzoxazone.

Results Two drugs fitted oral first orders absorption, one -compartment open model. The main parameters of the reference and test drugs were respectively as follow: Tpeak:1.48±0.41and 1.07±0.35h(0.01<p<0.05); Cmax:8.41±3.6 and 10.3±3.5 μg·ml⁻¹; T1/2:1.19±0.43 and 0.90±0.23h; AUC:25.0 and 24.3μg·h·mL⁻¹. The relative bioavailability of test drug is 99.08±20.59%

Conclusion Two chlorzoxazone tablets were bioequivalent.

Key words chlorzoxazone; pharmacokinetics; bioequivalence

Introduction
Chlorzoxazone (5-chloro-2(3H)-benzoxazolone, CZX) is a potent skeletal muscle relaxant that is effective in the treatment of painful muscle spasms. Following oral administration its effects begin within an hour and last for 3 to 4 hours. Chlorzoxazone exhibits minimal adverse effects and almost no gastrointestinal irritation[1].

Chlorzoxazone is reported to be completely absorbed after oral administration, and the peak serum concentrations are achieved after 1 to 2 h. The elimination half-life is about 1 h.[1] This paper presented the study on bioequivalence of chlorzoxazone tablet provided by Jinshi Pharmaceutical (Tianjin) Co., Ltd. compared with the product purchased from Zhejiang Yatai Pharmaceuticals in 8 healthy male volunteers following oral administration.

Subjects Selection
Eight normal, healthy, adult male subjects, age 20-21(20.1 ± 0.4 years old), height 170-178cm (174.9 ± 3.8cm), body weight 59.5-75kg (70.5 ± 5.8kg), participated in this study. They were found to be in good health as determined by a screening procedure consisting of physical examinations, evaluation of clinical laboratory values (hematology, blood chemistry, and urinalysis), and determination of standard ECG. None of the subjects had any serious diseases, and the refrained from taking drugs for a period of 2 weeks prior to this study.

All volunteers understood the study and signed the written-formed consent. Within two weeks the volunteers took no concomitant medication. The subject fasted for 12 hours. During the studying period, smoking, drink alcohol, tea were all prohibited and the subjects were under the supervision of medical staff.
Study protocol

This study was an open, single center, randomized crossed-over single dose study. Eight healthy male volunteers were divided to two groups randomly. In the morning one single dose of 400mg of test drug or 400mg of reference drug and 200ml of water were given orally to overnight fast subjects. Four hours later the subjects took a standard meal. One week later, the subjects received the second session of test drug or reference drug alternatively. During the whole study, the subjects were instructed by the investigator to report any adverse effects or events immediately. Venous blood samples were collected into heparinized tube pre-dose and then at 0.25, 0.5, 1, 1.5, 2, 3, 4 and 6 hours after dosing from the subjects. Blood samples were centrifuged 3000rpm for 10minutes at room temperature immediately after collection. Plasma samples were removed and transferred to tubes and frozen immediately at -20°C until determination.

Safety

Safety was evaluated by monitoring adverse events and vital signs, physical examinations, clinical laboratory tests and ECG. Each subject was questioned periodically throughout the study regarding adverse effects.

Materials

Drugs: test drug: chlorzoxazone 200mg/tablet, batch No.940920. Reference drug: chlorzoxazone 200mg/tab provided by Zhejiang Yatai Pharmaceuticals, batch No.940405. Standard chlorzoxazone provided by Jinshi Pharmaceuticals (Tianjin) Co. Ltd. Internal standard: phenacetin provided by Tianjin Li-Sheng Pharmaceuticals.

Disposable 1-mL C_{18} bonded-phase extraction columns (Hebei Jinyang filter works) were used to separate CZX from serum components. All other chemicals and solvents were the highest grade of commercially available materials.

HPLC conditions

The HPLC analyses were used. It consisted of Waters 510 pump, 710B injector, Shimadzu SPD-6A UV-visible detector and a C_{18} ODS column (250mm × 4.6mm I.D., 5μ m particle size). Data acquisition was accomplished with Waters 730 integrator. The mobile phase was methanol-0.05M sodium dihydrogen phosphate (50:50) at flow rate of 1.0ml·min^{-1}. The system was operated at ambient temperature, with UV detection at 280nm. Reference stock standard solutions were prepared in methanol.

Sample preparation

Plasma(0.5ml) and 10μ L of internal standard solution were mixed in an 1.5ml plastic centrifuge tube. And the 0.05M sulfuric acid (250μ L) were also added to each tube followed by mixing on a vortex mixer(30s). Each entire spiked plasma sample was quantitatively transferred to a disposable solid-phase extraction column. The extraction columns had been prerinsed with 5mL methanol followed by 5mL distilled water. The plasma sample and washing were drawn through the column. Then 5mL distilled water was drawn through the column. 1.5mL plastic centrifuge tubes were inserted into the manifold for collection of samples. The compound CZX and internal standard were then eluted from the column using 1mL methanol. The elution were stored at 4°C overnight and 20μ L sample was injected into the liquid chromatograph. Plasma concentration was calculated according to the peak-area ratios between the components and the internal standard.

Method validation

The Calibration curve of CZX in plasma was linear over the range 0.2-20.0 μ g·mL^{-1}, and the coefficient of correlation was 0.9998. The regression equation was Y=5.9766x -0.1669 (y: peak area ratio of I to internal standard, x: plasma concentration). The limit of detection was 0.2μ g·mL^{-1}, and the average recovery was 98.2± 5.5% over the range of 0.5-10 μ g/mL^{-1}. The precision of within-run assay and between-run assay were 2.4% and 3.47%, respectively (Table 1), and the coefficient of variation was within 10%. Fig 1 shows a chromatogram of the separation of CZX and internal standard in a plasma sample.

<table>
<thead>
<tr>
<th>Conc. in μ g·mL^{-1}</th>
<th>Within-run Recovery (%)</th>
<th>SD (%)</th>
<th>RSD (%)</th>
<th>Between-run Recovery (%)</th>
<th>SD (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>88</td>
<td>4</td>
<td>4.55</td>
<td>90</td>
<td>6</td>
<td>6.67</td>
</tr>
<tr>
<td>2.0</td>
<td>102.5</td>
<td>2</td>
<td>1.95</td>
<td>101</td>
<td>2</td>
<td>1.98</td>
</tr>
<tr>
<td>10.0</td>
<td>96.2</td>
<td>2.8</td>
<td>0.83</td>
<td>98.8</td>
<td>1.7</td>
<td>1.72</td>
</tr>
<tr>
<td>Mean</td>
<td>93.6</td>
<td>2.3</td>
<td>2.44</td>
<td>96.6</td>
<td>3.2</td>
<td>3.46</td>
</tr>
</tbody>
</table>

Pharmacokinetics parameters and statistic analysis
Pharmacokinetics parameters were calculated using 3p87 pharmacokinetics program. Peak plasma concentration (Cmax) and peak time (Tpeak) were taken directly from the observed data. The area under the plasma concentration-time curve (AUC0-6h) was calculated by the trapezoidal rule, observed in 8 normal subjects following the oral administration of 400mg of CZX.

![Blank plasma internal standard chlorzoxazone sample](image)

**Fig 1** The HPLC chromatography of plasma samples

Bioavailability evaluation was based on the area under curve (AUC). Formula: $F = (\text{AUC}_{\text{test drug}}/\text{AUC}_{\text{reference drug}}) \times 100\%$. Analysis of variance, two one-side tests and (1-2α) confidence interval were used to check bioequivalence of AUC(0-6h), AUC(0-6), Cmax, Tpeak and T1/2. The data were expressed by mean and standard deviation.

**Results**

Table 2 lists a summary $t_{1/2}, \text{Cmax}, \text{Tpeak}$, and AUC(0-6) observed in 8 normal subjects following the oral administration of 400mg of test drug/reference drug. The mean plasma $I$ concentration–time profile is shown in fig.2. test drug and reference drug were rapidly absorbed, the mean peak lever were $10.3 \pm 3.5$ and $8.41 \pm 3.6 \mu g \cdot mL^{-1}$, respectively; the mean peak time were $1.07 \pm 0.35$ and $1.48 \pm 0.41$ h, respectively after dosing. After reaching a peak, the plasma concentrations declined rapidly in a monoequivalent manner. Plasma concentra-tions at the 6 hr time points for most subjects were around the assay quantitation limit of $0.2 \mu g \cdot mL^{-1}$, indicating that CZX was rapidly eliminated from the body.

**Bioavailability and bioequivalence**

According to the above formula, the bioavailability of the test drug was $99.08 \pm 20.59\%$. RSD was $20.78\%$ (Table 3). AUC, Cmax and T1/2 of these two tablets were bioequivalent. Tpeak was not bioequivalent.

<table>
<thead>
<tr>
<th>Subject</th>
<th>AUC(test)(μg·mL⁻¹)</th>
<th>AUC(reference)(μg·mL⁻¹)</th>
<th>F(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30.96</td>
<td>32.05</td>
<td>96.6%</td>
</tr>
<tr>
<td>2</td>
<td>25.76</td>
<td>31.34</td>
<td>82.2%</td>
</tr>
<tr>
<td>3</td>
<td>17.95</td>
<td>20.42</td>
<td>87.2%</td>
</tr>
<tr>
<td>4</td>
<td>7.42</td>
<td>9.96</td>
<td>74.5%</td>
</tr>
<tr>
<td>5</td>
<td>35.33</td>
<td>25.24</td>
<td>140.0%</td>
</tr>
<tr>
<td>6</td>
<td>30.70</td>
<td>28.56</td>
<td>107.5%</td>
</tr>
<tr>
<td>7</td>
<td>32.89</td>
<td>35.64</td>
<td>92.3%</td>
</tr>
<tr>
<td>8</td>
<td>32.06</td>
<td>28.71</td>
<td>111.7%</td>
</tr>
<tr>
<td>Mean</td>
<td>26.63</td>
<td>26.49</td>
<td>99.1%</td>
</tr>
<tr>
<td>SD</td>
<td>9.45</td>
<td>8.10</td>
<td>20.6%</td>
</tr>
</tbody>
</table>

**Discussion and conclusion**

The method, using the solid-phase extraction and HPLC UV(280nm) detection, is simple and selective and enables the reliable quantitation of CZX. The result of validation showed the bioanalytical method was suitable for the bioavailability.[5,6]

The plasma concentration-time curve of 8 healthy volunteer shows that the absorption of the reference and test drugs was fast, it would reach the peak about one hour, and then distributed in vivo. The effective plasma concentration of drug declined
rapidly, the average excretions of 6 hours of two drugs were about 98.1% and 96.4% of the total amount of the drugs respectively.

Fig. 2. The mean plasma concentration-time curve
A: test drug  B: reference drug

There were no significant difference between AUC, Cmax and T1/2 of two drugs, but the Tpeak of test drug was faster than the reference one. It is suggest that the changes of two drugs within the body were basically same after absorption. The relative bioavailability of the test drug was 99.08 ± 20.59%, and RSD was 20.78%. The two tablets were bioequivalent.

References