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RESEARCH ARTICLE

The pharmacokinetics change of cefepime after Shuanghuanglian injection administration in subjects with the renal damage

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Abstract

Shuanghuanglian injection (SHLI) has been widely used for administration with cephalosporin in China for long time. The objective of this study was to evaluate the pharmacological properties and biochemical changes of cefepime combined with SHLI. The SD rats included were received either an intravenous (iv. 4 mL/kg) dose of normal saline, or intravenous (iv. 0.74, 0.37, 0.185 g/kg, respectively) doses of SHLI once daily for 7 days. After last administration, cefepime (0.41 g/kg) was intravenous injected to the animals. The serum and urine samples were acquired and stored at 4°C. They were used for quantitative determination of urea nitrogen (BUN), creatinine (CRE), urine protein, alkaline phosphatase (ALP) and N-acetyl-B-D-glucosaminidase (NAG). At different time points, the levels of cefepime in rat plasma were estimated for pharmacokinetic measures by HPLC. Aspirin was selected as internal standard (IS). The results showed that there were positive effects by increasing the total amount of CRE, BUN, NAG and urine protein (p<0.01 or <0.05) and decreasing the levels of ALP (especially the high dose group of SHLI with cefepime) (p<0.01). Besides, the pharmacokinetic results indicated that cefepime was distributed as non-compartment model after intravenous administration. Compared with the corresponding values for the compounds given alone, the area under the blood drug concentration time curve (AUC0–t and AUC0–1) was better increased in middle- and high-dose groups (p<0.01), the mean residence time (MRT) of cefepime was larger (p<0.01) and the total clearance (CL) was lower at different levels. The results mean that the duration and concentration of cefepime could be prolonged and the clearance reduced while in combination with SHLI. Furthermore, the cefepime in the three tested doses caused changes of renal tubular epithelial cells while the severity of changes mainly dependent on the specific doses. In conclusion, the results above-mentioned suggest a possible contribution of drug combination in the nephrotoxicity and biochemical alterations especially at high doses. Further, monitoring measures for the renal functions are warranted to evaluate during the combination of these two drugs.

Keywords

Cefepime, combination, nephrotoxicity, Shuanghuanglian injection

Introduction

Chinese herbal medicine (CHM) has been used as the medicine for health improvement or food supplements in China over thousand years. Historically, most of the CHM, with specific combination of different herbs as a formula, was prepared as decoctions by a unique methodology. Recently, a lot of CHM preparations have been produced for convenient to take. Among thousands of CHM preparations, Shuanghuanglian injection (SHLI) is one of the most widely used CHM preparations officially documented in the Chinese Pharmacopoeia, due to publicly-known antimicrobial properties (The State Pharmacopoeia Commission of PR China, 2010). SHLI was extracted from three raw medicinal herbs, Lonicera japonica Thunb. (Jinyin Hua in Chinese), Scutellaria baicalensis Georgi (Huangqin in Chinese) and Forsythia suspensa (Thunb.) Vahl (Lianqiao in Chinese), after a series of protocols, such as extraction, purification, spraying and sterilized packing. Since it was first approved for market in 1992 (Chen et al., 2002), SHLI has been commonly used for treating various kinds of infectious diseases caused by bacterium or viruses in respiratory tract infection. Numerous studies have shown that SHLI possessed obvious virostatic and bacteriostatic activities against certain viruses and bacteria, such as influenza, respiratory syncytial virus, hemolytic streptococcus and even against Pseudomonas aeruginosa, which can cause severe infections (Zhang et al., 2013; Zu et al., 2010). Besides, SHLI, which is the freeze-dried powder of SHLI, is considered as more stable and easy to preserve.

Cefepime, a semi-synthetic fourth-generation cephalosporin, is currently widely used in hospitals for its approved indications. It has in vitro activity against Gram-positive

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organisms including Staphylococcus aureus and penicillin-sensitive, -intermediate and -resistant Streptococcus pneumoniae similar to that of cefotaxime and ceftriaxone (Alfandari et al., 2007; Mutnick et al., 2004; US Food and Drug Administration, 2007). Moreover, cefepime also has good activity against Gram-negative organisms including P. aeruginosa, similar to that of ceftazidime (Chapman & Perry, 2003).

Nowadays, more preparations of CHM are paired with other drugs (including western medications) in Chinese hospital. Many kinds of CHM combined with western medications were usually given to patients. However, without strict clinical compatibility experience, severe medicinal accidents could be caused by such drugs in combination. Among those, the pharmacokinetic drug–drug interaction (DDI) is one of the most unfavorable clinical events caused by abnormal increasing or decreasing of drug concentrations in the human body while the co-administration of other drugs were taken.

One research with a total of 4382 medical records found that SHLI was commonly used in the form of combination, including 82.79% accounted for the combination with antibiotics and only 1.03% used the medicine alone (Wu et al., 2004; Zhang et al., 2010a). Among those, SHLI was usually administered with cefepime (Wang et al., 2012). Besides, many researches have been carried out, such as detection methods, pharmacokinetic behavior and evaluation of animal models (Han et al., 2014; Yan et al., 2013; Zhang et al., 2010b).

Our preliminary study has showed that SHL combined with cefepime will promote the incidence of renal pathological changes while compared with that administered with cefepime alone. We guess SHLI might change pharmacological properties of cefepime to produce renal pathological change (Towne et al., 2009). Furthermore, we hope to observe sensitive and reliable early indicators to evaluate the effects on renal tubular epithelial cell. However, the absolute bioavailability and the possible interactions between SHL and other antibiosis drugs on the pharmacokinetics have not been reported recently. The effects on renal tubular cell have never been studied yet.

On the other hand, there are many reports about adverse drug reactions (ADRs) of Shuanghuanglian (Wang et al., 2010). Whether the ADRs were caused by combination is still remain uncertain. The aim of the present study was to further investigate the pharmacological and biochemical changes of cefepime while SHLI administrated in combination with other medications and to provide references for the safety evaluation.

Materials and methods

Materials

Cefepime hydrochloride for injection was purchased from Luoxing Group Ltd (Shandong, China). SHLI powder for injection was provided commercially from pharmacy, which was made from the Second Chinese Medicine Factory, Harbin Pharm Group Co. Ltd. (Heilongjiang, China). Cefepime and aspirin (all purity &gt;98.0% as shown by HPLC) (Figure 1) were purchased from the National Institute for Food and Drug Control (Beijing, China). Methanol and acetonitrile were of HPLC grade and purchased from TEDIA Company Inc. (Fairfield, OH). Creatinine (CRE), urea nitrogen (BUN), N-acetyl-B-D-glucosaminidase (NAG), alkaline phosphatase (ALP) and urine protein diagnostic reagents were purchased from Nanjing Jiancheng Group Ltd (Nanjing, China). All other chemicals were of analytical grade and used without further purification. Double distilled water, prepared in our laboratory, was used throughout the study.

Animals

Sprague-Dawley (SD) rats (weight 250 ± 20 g) were purchased from the Medical Experimental Animal Center of Guangdong Province, China (Guangzhou, China). Animals were housed under the controlled standard conditions (22 ± 2°C, relative humidity 50 ± 20%) with the natural light–dark cycle and with free access to standard rat food (Laboratory Rodent Chow) and water for at least one week prior to experiment. And all animals were put into the metabolize cage to collect the urine of 24 h in same conditions. The animals were fasted overnight with free

![Figure 1.](H. Chen et al. Drug Chem Toxicol, 2016; 39(2): 129–136)
access to water. All animal experiments were performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of TCM University.

All animals were randomly assigned to four groups: a control group (0.9% sodium chloride only) and three dosed groups, low-, middle- and high-dose groups (0.74, 0.37, 0.185 g/kg, which were 2 -, 1 - and ½-fold of clinical dosages, respectively) to diminish the individual variation.

**Instruments**

Clinical chemistry analysis of serum samples was carried out with Automatic Biochemistry Analyzer (ECHO, Italian). The assay was performed on an Agilent 1100 series HPLC system (Agilent Technologies Inc., Santa Clara, CA) consisting of a quaternary pump, a diode array detector (DAD), an autosampler, a vacuum Degasser and a column oven.

**Preparation of standard and quality control (QC) samples**

Stock solutions of cefepime were prepared in methanol–water (10:90, v/v) at concentration of 1 mg/mL. A series of mixture (IS) stock solution was prepared in methanol–water (10:90, v/v) at concentration of 1 mg/mL. A series of mixture standard working solutions with concentration 0.5–3000 μg/mL for cefepime were obtained by diluting the mixture of the stock solution with methanol–water (10:90, v/v). The stock solution of IS was diluted to concentration of 200 μg/mL with methanol–water (10:90, v/v) as working solution. All the solutions were stored at 4 °C.

Working solutions of cefepime were prepared daily by appropriate dilution in acetonitrile. Primary stock solution of cefepime and aspirin was prepared by dissolving accurately weighed cefepime and aspirin in acetonitrile to yield a final concentration of 0.125 and 750 μg/mL, respectively. Plasma calibration standards (0.125, 1.25, 2.5, 5, 12.5, 25, 50, 125, 250, 625, 750 μg/mL) were prepared by spiked 50 μL cefepime working solution and 50 μL IS to 150 μL blank plasma.

Three levels of QC samples (5, 25, 125 μg/mL) in plasma were prepared separately in the same fashion.

**Preparation of samples**

SHLI powder for injection was dissolved in 0.9% normal saline. The control group of rats were administered an intravenous dose of normal saline (4 mL/kg), and the other three groups were given SHLI powder for injection at intravenous dose of 0.74, 0.37, 0.185 g/kg, respectively for 7 days. After 0.5 h of last administration, cefepime (0.41 g/kg) was intravenously injected to the rats. And all drugs were intravenously injected though caudal vein.

Blood samples were collected at 0.033, 0.117, 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8 h after intravenous administration. Blood samples taken from orbital vein were collected in heparinized 1.5 mL polytubes before dosing and at each collecting points. Then the samples were immediately transferred into heparinized tubes to separate the plasma by centrifuged at 12000g for 15 min at 4 °C. Stock solutions were prepared in methanol and stored at −80 °C when not in use.

For biochemical analysis, urine of 24 h was collected by using the metabolize cage examination. After animals were anesthetized by 10% chloral hydrate, blood samples of abdominal aorta were collected. The collected sera were centrifuged (3000g, 10 min), and then the supernatants were transferred into tubes. These supernatants and urine samples were stored at −80 °C until analysis.

**Sample determination**

Frozen samples for biochemical analysis were thawed at room temperature and determined by Automatic Biochemistry Analyzer for NAG, ALP and urine protein.

Other samples were thawed at room temperature and treated as follows. To an acidified rat plasma sample (150 μL) in a 1.5 mL eppendorf tube was added 50 μL methanol–water (10:90, v/v) and aspirin, respectively. The mixtures were vortexed for 5 min, and then added with 50 μL of 3.70 M perchloric acid–water (30:70, v/v). At last, the mixtures were vortexed for 5 min and centrifuged at 12 000g for 15 min. The supernatant was clear and do not need to be filtrated. An aliquot of 150 μL supernatant was transferred to an auto-sampler vial and 20 μL was injected into the HPLC column.

**HPLC instrument and analytical conditions**

All analyses were performed on an Agilent 1100 series HPLC system (Agilent Technologies Inc.) equipped with a binary solvent manager, sample manager, column compartment, ultraviolet detector and LC-Solution software. The separation was performed on a Phenomenex C18 column (Luna, 250 mm × 4.6 mm) (Phenomenex, Torrance, CA). The detection wavelength was set at 254 nm. The mobile phase consisted of acetonitrile (A) and 0.05% methanolic acid (B). The linear gradient was as follows: 0–4 min, 7% A; 5–11 min, 35% A; 12–14 min, 7% A, at a flow rate of 1.0 mL/min (Table 1). The column temperature was maintained at 25 °C and the injection volume was 20 μL.

**HPLC method validation**

**Specificity**

The specificity was evaluated by comparing chromatograms of blank plasma (from six different rats), blank plasma spiked with cefepime and aspirin, and plasma samples obtained from rats administrated by SHLI and/or cefepime. As presented in Figure 1, no significant endogenous peak interfering with cefepime and aspirin was obtained in blank plasma.

**Standard curve and sensitivity**

Selectivity was tested by comparison of blank plasma from six individual rats with corresponding spiked plasma samples.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Flow rate (mL/min)</th>
<th>A%</th>
<th>B%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td>12</td>
<td>1</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>14</td>
<td>1</td>
<td>7</td>
<td>93</td>
</tr>
</tbody>
</table>

A – acetonitrile, B – 0.05% formic acid.
Calibration curves were constructed from the peak–area ratios of each analysis to aspirin versus plasma concentrations using a 1/\(X^2\) weighted linear least-squares regression model. The lower limit of quantification (LLOQ) was defined as the final concentration producing a signal-to-noise (S/N) ratio larger than 10.

Intra-day and inter-day precisions were evaluated by measured results of QC samples at low, medium and high concentrations. Accuracy was determined as the percentage deviation of the mean detected concentrations from the nominal concentrations. Extraction recoveries were determined by comparing the peak area obtained from plasma sample spiked before extraction with those from plasma samples spiked after extraction. The matrix effect was investigated by comparing the absolute peak area of control plasma extracted and then spiked with a known amount of drug to that of neat standard samples at equivalent concentrations. The same procedure was performed for stability of samples and was assessed by analyzing samples stored at \(-20^\circ\)C for 1 month, subjected to three freeze (\(-20^\circ\)C to thaw (room temperature) cycles, and stored at room temperature for 4h. Samples were considered stable with the deviation from the nominal concentration within \(\pm 15.0\%\).

**Assay application**

The WinNonlin Standard Edition, Version 1.1 (Scientific Consulting Inc., Apex, NC) was used to calculate pharmacokinetic data. An IV-bolus input non-compartmental model was used to find the blood pharmacokinetic parameters, including elimination half life \((t_{1/2})\), mean residence time of drug \((\text{MRT})\), area under the concentration–time curve \((\text{AUC}_0\text{–}_{t})\) and \((\text{AUC}_0\text{–}_{\infty})\), clearance \((\text{CL})\) and the apparent volume of distribution \((V_d)\). The results were presented as mean \(\pm\) standard deviation.

**Statistical analysis**

Analysis was performed by Student’s \(t\)-test and one-way ANOVA with post-hoc test using SPSS programs, version 13.0, while a \(p<0.05\) was considered statistically significant and \(p<0.01\) was highly significant.

**Results**

**Method validation**

**Specificity**

The specificity was evaluated by comparing chromatograms of blank plasma (from six different rats), blank plasma spiked with cefepime and aspirin, and plasma samples obtained from rats administrated by SHLI. As presented in Figure 1, no significant endogenous peak interfering with cefepime and aspirin was obtained in blank plasma. The results showed that cefepime in rat plasma were detected 6 min after administration of the SHLI and cefepime, respectively.

**Linearity for calibration curves and lower limit of quantification**

The linearity, limit of detection (LOD) and limit of quantity (LOQ) of the proposed method were evaluated. Working solutions of cefepime were prepared daily by appropriate dilution in acetonitrile. Primary stock solution of cefepime and aspirin was prepared by dissolving accurately weighed cefepime and aspirin in acetonitrile to yield a final concentration of 0.125 and 750 \(\mu\)g/mL, respectively. Plasma calibration standards (0.125, 1.25, 2.5, 5, 12.5, 25, 50, 125, 250, 625, 750 \(\mu\)g/mL) were prepared by spiked 50 \(\mu\)L cefepime working solution and 50 \(\mu\)L I.S. (200 \(\mu\)g/mL) to 150 \(\mu\)L blank plasma.

The method was linear over the concentration range of 0.125–750 \(\mu\)g/mL for cefepime, a signal-to-noise (S/N) >3 at the LOD was observed and the LOD was 0.037 \(\mu\)g/mL. Linear calibration curves equation was: \(Y = 2.9853X - 0.2672\), with correlation coefficient \(r\) was 0.9998 (Figure 2). These limits were sufficient for the pharmacokinetic studies of analyses.

**Precision and accuracy**

The values for intra-day and inter-day precision and accuracy for the plasma sample are shown in Table 2. For each sample, it could be seen that RSDs of inter-day and intra-day are less than 5% and the average recovery of the method was higher than 90%, respectively. The values indicated an acceptable precision and accuracy for the present method.

**Stability**

The stability of analyte in rat plasma was assayed including autosampler for 12h, freeze and thaw for three times and long-term stability at \(-80^\circ\)C for 30 days. The results are summarized in Table 3. The data indicated that the five samples were all stable in plasma for three freeze/thaw cycles, auto-sampler for 12h at room temperature and freeze and thaw for three times, and long-term stability at \(-80^\circ\)C for 30 days.

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**Figure 2. Standard curve of cefepime in rat plasma.**

**Table 2. The accuracy, precision and recovery rate of cefepime in rat plasma \(\overline{X} \pm s, N = 5\).**

<table>
<thead>
<tr>
<th>Compounds concentration (mg/L)</th>
<th>Intra-day (\text{RSD%})</th>
<th>Inter-day (\text{RSD%})</th>
<th>The recovery of the method (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2.97</td>
<td>3.43</td>
<td>89.4 (\pm) 0.62</td>
</tr>
<tr>
<td>25</td>
<td>3.67</td>
<td>3.92</td>
<td>99.7 (\pm) 0.98</td>
</tr>
<tr>
<td>125</td>
<td>3.35</td>
<td>2.87</td>
<td>90.8 (\pm) 1.03</td>
</tr>
</tbody>
</table>

**Table 3. Summary of long-term stability at \(-80^\circ\)C for 30 days.**

<table>
<thead>
<tr>
<th>Compounds concentration (mg/L)</th>
<th>RSD%</th>
<th>Recovery of the method (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>5.0</td>
<td>89.4 (\pm) 0.62</td>
</tr>
<tr>
<td>25</td>
<td>5.2</td>
<td>99.7 (\pm) 0.98</td>
</tr>
<tr>
<td>125</td>
<td>4.5</td>
<td>90.8 (\pm) 1.03</td>
</tr>
</tbody>
</table>
**Pharmacokinetic study**

In this study, the validated analytical method was employed to determine the concentration of cefepime. The cefepime concentration in plasma was in conformation with a non-compartment mode by using the WinNonlin software. The mean pharmacokinetic parameters were calculated and shown in Table 4. Plasma concentration–time profiles of cefepime after intravenous administration are shown in Figure 3 with corresponding pharmacokinetic parameters given in Table 4. After intravenous administration of SHLI at doses of 0.185, 0.37, 0.74 g/kg day\(^{-1}\) for 7 days to rats, the mean plasma concentration–time profiles of cefepime are shown in Figure 3. According to results, the pharmacokinetic of cefepime was best fitted to a non-compartment model.

**Biochemical samples analysis**

From Table 5, SHLI at the three administered doses with cefepime induced significant (\(p<0.01\)) increase in NAG and urine protein concentration in serum together with significant (\(p<0.01\)) decrease in its ALP concentration by contrast with the blank control group and the cefepime group, except urine protein of cefepime contrast with the blank control group.

From Table 6, serum CRE and BUN levels were significantly elevated in all combination groups (\(p<0.01\) or 0.05) by contrast with the blank control group and the cefepime group, and cefepime can enhance the level of CRE contrast with the blank control group.

Table 3. The stability of cefepime in rat plasma (\(\bar{x} \pm s, N = 5\)).

<table>
<thead>
<tr>
<th>Compound concentration ((\mu g/mL))</th>
<th>Freeze–thaw cycles</th>
<th>Mean ((\mu g/mL))</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>12 h room temperature</td>
<td>3.73 ± 0.08</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td>–20°C freeze/thaw</td>
<td>3.91 ± 0.11</td>
<td>2.91</td>
</tr>
<tr>
<td></td>
<td>–80°C 30 days</td>
<td>4.28 ± 0.19</td>
<td>4.60</td>
</tr>
<tr>
<td></td>
<td>12 h room temperature</td>
<td>19.6 ± 0.33</td>
<td>1.73</td>
</tr>
<tr>
<td>25</td>
<td>–20°C freeze/thaw</td>
<td>19.9 ± 0.49</td>
<td>2.51</td>
</tr>
<tr>
<td></td>
<td>–80°C 30 days</td>
<td>19.9 ± 0.40</td>
<td>2.03</td>
</tr>
<tr>
<td></td>
<td>12 h room temperature</td>
<td>108 ± 1.02</td>
<td>5.71</td>
</tr>
<tr>
<td>125</td>
<td>–20°C freeze/thaw</td>
<td>107 ± 4.20</td>
<td>3.92</td>
</tr>
<tr>
<td></td>
<td>–80°C 30 days</td>
<td>111 ± 1.92</td>
<td>1.74</td>
</tr>
</tbody>
</table>

Table 4. Pharmacokinetic parameters of cefepime single dose after SHLI intravenous injection for a week once a day (\(\bar{x} \pm s, N = 8\) or \(N = 7\)).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cefepime (0.410 g/kg)</th>
<th>SD (0.740 g/kg (\cdot d^{-1})) + cefepime (0.410 g/kg)</th>
<th>SZ (0.370 g/kg (\cdot d^{-1})) + cefepime (0.410 g/kg)</th>
<th>SG (0.185 g/kg (\cdot d^{-1})) + cefepime (0.410 g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUC(0–(t)) ((\mu g h/mL))</td>
<td>395 ± 30.0</td>
<td>402 ± 30.7</td>
<td>439 ± 21.9**</td>
<td>465 ± 30.3**</td>
</tr>
<tr>
<td>AUC(0–(\infty)) ((\mu g h/mL))</td>
<td>396 ± 29.8</td>
<td>405 ± 30.0</td>
<td>441 ± 22.6**</td>
<td>469 ± 29.4**</td>
</tr>
<tr>
<td>MRT (0–(\infty)) (h)</td>
<td>0.770 ± 0.020</td>
<td>0.970 ± 0.020**</td>
<td>1.00 ± 0.040**</td>
<td>1.05 ± 0.040**</td>
</tr>
<tr>
<td>(t_1/2) (h)</td>
<td>1.38 ± 0.470</td>
<td>1.67 ± 0.570</td>
<td>1.43 ± 0.340</td>
<td>1.68 ± 0.460</td>
</tr>
<tr>
<td>(T_{\text{max}}) (h)</td>
<td>0.033</td>
<td>0.033</td>
<td>0.033</td>
<td>0.033</td>
</tr>
<tr>
<td>(V_d) (L/kg)</td>
<td>2.09 ± 0.790</td>
<td>2.47 ± 0.910</td>
<td>1.91 ± 0.410</td>
<td>2.12 ± 0.660</td>
</tr>
<tr>
<td>(CL) (L/h/kg)</td>
<td>1.04 ± 0.080</td>
<td>1.02 ± 0.070</td>
<td>0.930 ± 0.050</td>
<td>0.88 ± 0.050*</td>
</tr>
</tbody>
</table>

*With blank control group: \(p < 0.05\).
**With blank control group: \(p < 0.01\).

**Discussion**

The selection of internal standard was also investigated in this study. Based on the chemical property of cefepime, aspirin was tested. The internal standard showed the suitable retention time and no interference from the endogenous matrix. Therefore, aspirin was selected as internal standards to determine cefepime.

SHLI is a commonly used CHM preparation which has been used largely for millions of patients with respiratory infections since 1973. Recent studies indicate that the antimicrobial properties of SHLI can be predominantly attributed to its effective ingredients, such as caffeotannic acid, saponins and baicalin (Fattouch et al., 2007; Kong et al., 1993; Pasi et al., 2009). And many investigation of toxicological effects of SHLI have been reported (Yan et al., 2012).

More and more preparations of CHM have been paired with other drugs (including western drugs) in Chinese hospital. Even if without prescription, some patients were given other kinds of drugs for coexisting diseases. The incidence of ADR from drug–drug interaction increases significantly. It has been reported that cephalosporin is one of the most common combination choice with SHLI from data include 600 cases of 48 studies (Wang et al., 2012). After combination, higher ADR risk opportunities appeared. Our study indicated that SHLI can increase the AUC of cefepime significantly, and this might increase the opportunities of ADR.

The AUC\(_{0-\infty}\) and AUC\(_{0-\infty}\) of cefepime single dose after SHLI (0.74, 0.37 g/kg day\(^{-1}\)) intravenous administration for 7 days are all significantly higher than that of corresponding

![Figure 3. Mean T–C plot of cefepime single dose after SHLI intravenous injection for a week once a day on SD rats.](image-url)
Cefepime administration only (p all <0.01), and the rates of increase are 17.7% and 11.1%, respectively. We found that the higher the dosages of SHLI, the higher AUC was found in this study, which might provide further information regarding the herbal drug extract interaction between SHLI and cefepime. Administration of SHLI combined with cefepime single dose each gave higher MRT values than pure SHLI. This reflects the much greater retention time of cefepime because of SHLI. CL of cefepime was estimated to be 0.88–1.04 h/L, and the value of combination with SHLI (0.74 g/kg d−1) decreased significantly (p > 0.05). That means the combination can prolong the duration of cefepime in body. Our other studies have indicated that SHLI combined with cefepime will promote the activity of organic anion transporter 1 (OAT-1), which is an important renal transporter to transport anion from extracellular to intracellular in basal lateral, and cefepime is its substrate (Hao et al., 2012). Further research about other transporters and its mechanism for effect is underway.

On the other hand, there were no significant differences in $V_d$ values after iv. of SHLI for 7 days and single cefepime. The $V_d$ values of 2.09, 2.12, 1.91, 2.07 L/kg were three times more than that of whole body water in rat (0.668 mL/kg) indicating extensive tissue distribution. It has been verified that cefepime has wide distribution in body tissues and fluid (Gutierrez, 2004).

Tissue distribution of cefepime was rapid as indicated by the short distribution half-life of $(t_{1/2})$ of 1.43–1.68 h while $(t_{1/2})$ of cefepime was only 1.38 h. That means SHLI combined with cefepime single dose can increase the distribution time in body, in some degree.

Cefepime ($C_{19}H_{24}N_6O_5S_2$) is a newer semi-synthetic fourth-generation cephalosporin which has excellent activity against both Gram-positive as well as Gram-negative organisms (Figure 4). It is a white to pale yellow powder and is highly soluble in water (Capparelli et al., 2005; Gutierrez, 2004; Kessler et al., 1985; Shahid, 2008).

Cefepime is used at doses of 2–4 g/dose one day in adult (maximum of 6 g/day). At these doses, effective and non-toxic serum cefepime levels are reached (Capparelli et al., 2005; Shahid, 2008). But this did not mean it has no any potential bad effect (Fang et al., 2006; Zhou et al., 2010). When cefepime was combined with other drugs, such as β-lactam antibiotic, side effects arised. Cefepime is eliminated by kidney. If it is hindered from leaving out of body, it will gather in kidney. That will incur renal toxicity.

Kidney is a common target of toxicity of therapeutic and environmental xenobiotics, because of its high blood flow, tubular transport processes and complex metabolic activities. As cefepime is excreted by the kidney, it might result in nephrotoxicity, especially producing the acute proximal tubule necrosis in both animals and human (Kamiya et al., 1983; Kuo & Hook, 1982; Tune, 1997). The nephrotoxicity of cephalosporins is dependent upon renal cortical accumulation and intracellular concentration (Elsayed et al., 2014; Mossad et al., 2014).

As Servais reported, cefepime was more toxic when used in combination with other medications (Khalili et al., 2013; Mossad et al., 2014; Servais et al., 2005). The traditional laboratory approach for detection of renal disease does not allow for early detection of acute renal injury. Damage to renal tubules can be insufficient to result in a change in a parameter of kidney function, such as serum CRE and BUN. In addition, in case of more extensive tubular injury, there is a lag in time between the injury and an increase in serum CRE. Sensitive and reliable biological markers of renal tubular injury, such as NAG, are needed in order to detect early kidney injury which has been generally accepted (Yamashita et al., 2014).

CRE and BUN are the most important parameters to measure the function of kidney. Combination cefepime and

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (g/kg)</th>
<th>NAG (U/L)</th>
<th>Urine protein (µg/mL)</th>
<th>ALP (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>0.400 mL/100 g</td>
<td>49.8 ± 6.55</td>
<td>528 ± 52.9</td>
<td>212.0 ± 69.3</td>
</tr>
<tr>
<td>Cefepime</td>
<td>0.410</td>
<td>72.6 ± 9.75</td>
<td>648 ± 89.5</td>
<td>103.0 ± 28.4</td>
</tr>
<tr>
<td>SG + cefepime</td>
<td>0.740 ± 0.41</td>
<td>127.0 ± 11.2</td>
<td>1339 ± 191</td>
<td>98.3 ± 24.4</td>
</tr>
<tr>
<td>SZ + cefepime</td>
<td>0.370 ± 0.41</td>
<td>107.0 ± 13.2</td>
<td>1127 ± 227</td>
<td>125.0 ± 37.6</td>
</tr>
<tr>
<td>SD + cefepime</td>
<td>0.185 ± 0.41</td>
<td>101.0 ± 12.7</td>
<td>1110 ± 162</td>
<td>110.0 ± 28.3</td>
</tr>
</tbody>
</table>

*With blank control group: p < 0.05.
**With blank control group: p < 0.01.
ΔWith cefepime group: p < 0.05.
ΔΔWith cefepime group: p < 0.01.

Figure 4. Chemical structure of cefepime.
SHLI caused a marked reduction in renal function, as characterized by significant increases in the plasma CRE concentrations and blood BUN concentrations, when compared with the control group (Table 6). Thus, these data indicated that a single large dose intravenous injection of these two drugs can impair renal function in a short time. The increased CRE and BUN levels in the blood suggested diseases and conditions that affected the kidney function (Courrèges et al., 1998). Meanwhile, we found that cefepime intravenous injected for 7 days will significantly promote the level of serum CRE. These findings provided the initial evidence that cefepime indeed induced renal damage.

Combination of cefepime and SHLI caused increased urinary NAG level. This might be attributed to the progressive cellular and tubular dysfunction manifested histopathologically (Ali et al., 2014). The kidney function can be evaluated by a number of methods, including the assessment of urinary enzymes. NAG is a lysosomal enzyme that is present in proximal tubular cells. It is stable against changes in pH and temperature. The NAG has a relatively high molecular weight of approximately 130,000–140,000 Da which does not permit its filtration through the glomerular basal membrane and it is rapidly cleared from the circulation by the liver. Thus, urinary NAG originates primarily from the proximal tubule, and increased urinary excretion is a consequence of renal tubular cell breakdown, especially in acute kidney injury (Skalova et al., 2009). Now, NAG has been remarked to be one of the most sensitive biological markers of renal tubular injury.

ALP is one of the enzymes associated with renal function. ALP decrease is usually associated with damage of kidney, especially in basement membrane. Basement membrane was essential to renal tubular cells (Adeyemi et al., 2010). When drug destroyed the integrity of plasma membrane, renal function will change. Biochemical alterations observed were complemented by changes in the kidney. Combination cefepime and SHLI will change the activity of transporters, for example, the OAT-1. And that might be associated with cefepime-induced renal damage.

Combination of cefepime and SHLI significantly elevated urine protein content. This might be attributed to the failure of renal glomerulus and tubules. After pathological changes appeared and influenced the function of glomerulus or tubules, large protein gathered to form cylindruria. This was also consistent with other researches following administration of cefpirome, urinary protein was detected (Deki et al., 1990).

As we have reported, histopathological changes in the kidney after intravenous injection of cefpime and/or SHLI for seven consecutive days in rats were investigated (Hao et al., 2012). And the changes were dose dependent. All results above were agreed with the histopathological fundings.

Conclusions

Combination of cefepime and SHLI could effectively suppress renal ALP activities, and markedly increase the renal CRE, BUN, NAG and urine protein level. All these results were in accord with the histopathological fundings we have reported (Hao et al., 2012).

Based on the research results, we found that there could be a significant drug–drug interaction between SHLI and cefepime. Clinical doctors should take the failure or ADR into consideration with caution while considering the combination of these two drugs. Further detailed information on the integrative treatment, i.e. CHM combined with western medications was attempted to provide for the decision-making of clinical practice.

Declaration of interest

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All authors declared that there were no conflicts of interest.

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