A pharmacokinetic study of ceftazidime by using high pressure liquid chromatography in normal Thai volunteers.

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Ceftazidime is a semisynthetic third-generation cephalosporin with a broad spectrum of activity. It is highly active, particularly against Pseudomonas spp. which is one of the most important causation for infectious diseases. The purpose of this study is to describe a High-pressure liquid chromatography (HPLC) assay for the determination of ceftazidime concentration in serum and urine in 8-volunteers. The volunteers were administered ceftazidime pentahydrate 1 gm (equivalent to ceftazidime base 757.33 gm) by intravenous injection at the right antecubital vein. Five milliliters of blood were collected at 0.5,15,30,45 minutes after that and at 1,2,4,6,8,12 and 24 hours respectively. Urine samples were collected at 0-2, 2-4, 4-6, 6-8, and 12-24 hours. All serum and urine samples were analysed for determination of ceftazidime concentration by using HPLC method. The result of the study revealed that the cefazidime half life in serum and in urine were 1.26±0.19 and 1.40±0.17 hours respectively. These differences were not statistically significant. The serum half life from Thai volunteers were similar to other studies from abroad. The HPLC assays presented a rapid, sensitive and reproducible method for the detection of cefazidime concentration in both serum and urine.

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Received for publication. October 9, 1990.

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ดูผ่าชิ้นป้องกันเฉพาะพืชในสูติวิทยา 79.3 ความผ่าชิ้นป้องกันที่สำคัญ ต้องการปรับปรุงการชักเสียบกลุ่ม Pseudomonas aeruginosa ซึ่งเป็นสาเหตุสำคัญในโรคพืชชื้น ในการศึกษาได้จำแนกชีวิต high pressure liquid chromatography (HPLC) มาใช้ในการศึกษาความตระกูลอย่างที่ได้ทำไว้ในกลุ่มพืชในกลุ่มพืชชื้น 8 ชนิด ไล่จากชิ้นป้องกัน 1 กิโลกรัม (เท่ากับชิ้นป้องกัน 75.33 กรัม) จนถึงชิ้นป้องกันและจะเลือกจำนวน 5 กรัม แต่ละชนิด แต่ละชนิดได้ทำป้องกันเยื่อ 0, 5, 15, 30, 45 กรัม และ 1, 2, 4, 6, 8, 12, และ 24 ชิ้นต่อตามลำดับ จำนวนนั้น กับการเยื่อป้องกัน 1 คิวสิ่งไม่ต้องการ ต้องการวิเคราะห์ HPLC ของกลุ่มพืชในกลุ่มที่มีชีวิต mean half life ได้จากชิ้นป้องกัน 1.26 ± 0.19 ชั่วโมง และในกลุ่มป้องกันตัวตัว 1.40 ± 0.17 ชั่วโมง ตามลำดับ โดยการวิเคราะห์ได้จากการวิเคราะห์ในกลุ่มพืชและป้องกัน แต่กลุ่มตัวตัวอย่างไม่มี นัยสำคัญทางสถิติ และตัวตัวชิ้นป้องกันได้จากการวิเคราะห์ในกลุ่มพืชและป้องกันไม่มีนัยสำคัญทางสถิติ เทียบกับ การวิเคราะห์ข้อมูลจากชิ้นป้องกันโดยใช้ HPLC ที่มีข้อมูลที่มีความน่าเชื่อถือและความถูกต้องสูงประการพืชผ่านวิธีการ ไม่มีข้อขัดใจในการเทียบชิ้นป้องกันไว้ในกลุ่มพืชและป้องกัน
Ceftazidime is a semisynthetic third-generation cephalosporin with a broad spectrum of activity. It is highly active, particularly against Pseudomonas spp., Serratia spp., and indole-positive Proteus spp., organisms which have been resistant to earlier cephalosporins. It is highly stable to Beta-lactamases from gram-negative organisms and to staphylococcal penicillinas (1).

For proper use of the drug in treating serious bacterial infections in patients at the hospital, a thorough understanding of the biodisposition and pharmacokinetics of ceftazidime is mandatory. Traditionally, microbiological assays have been used to determine antibiotic concentrations in biological fluids. Even though this method is sensitive, it has a high capacity for processing samples, and measures only the biologically active component in the test system; the lack of specificity, limited reproducibility and an inability to detect biologically inactive metabolites are major disadvantages. An additional disadvantage is the length of time required for sample analysis. High pressure liquid chromatography (HPLC) provides a sensitive and specific alternative with the additional advantages of high precision and rapid turn-around time. The purpose of this report is to describe an HPLC assay for the determination of ceftazidime concentration in serum and urine in 8-volunteers.

Material and Method

Subjects and Trial design

Eight volunteers from the gynecology ward, Chulalongkorn hospital, were recruited into the study from March - June 1989. They were admitted in the hospital for operations due to medical indications including, endometriotic cyst, recurrent dysfunctional uterine bleeding (D.U.B.), myoma uteri, and ovarian cyst. All volunteers signed a consent form to participate in the study after being fulling informed of the trial procedures. History taking, physical examination and laboratory investigations were performed for each volunteer on her admission. The following laboratory investigations were done, complete blood count, urinary analysis, liver function test, and renal function test. All subjects did not receive any drugs one week before entering in this study.

Twenty-four hours before the operation, volunteers were transferred to the metabolic ward in the Obstetric-Gynecology department. All emergency kits were prepared during the study. The procedures were as follows:

1. Volunteers were administered with ceftazidime pentahydrate 1 gm (equivalent to ceftazidime base 757.33 gm) by intravenous injection at the right antecubital vein over a 3 minutes period.
2. Blood samples were taken at the left antecubital vein by a heparin lock needle. Five milliliters of blood were collected at 0, 5, 15, 30, and 45 minutes after that at 1, 2, 4, 6, 8, 12 and 24 hours respectively. Serum was separated by centrifuging technique and was stored frozen at -20°C.
3. Urine samples were collected at 0-2, 2-4, 4-6, 6-8, 12-24 hours by measuring all total volumes, and only twenty milliliters each was frozen at -20°C.

All serum and urine samples were prepared for the HPLC determination of Ceftazidime concentration the following day.

Assay

Reagent

Ceftazidime pentahydrate was donated by Glaxo Thailand. The internal standard, cefalexin, was purchased from Istituto Biochimico Italiano. Perchloric acid and Formic acid were reagent grade and from Carlo erba. Acetonitrile (HPLC grade) was purchased from Koch-Light Limited. Ammonium dihydrogen phosphate came from Riedel-de haen A.G. Seelze-hannover.

Sample preparation

250 µl volume of serum was extracted by adding 250 µl volume of working standard solution which consisted of internal standard (equivalent to 50 mg/L of Cefalexin) and perchloric acid 70%. The mixture was then blended with a vortex mixer for 5 seconds and centrifuged at 12,800 xg for 5 minutes (4,5) A 10 µl volume of the supernatant fluid was then injected through a U6K valve injector.

Urine sample was prepared for injection by sedimenting particulate matter with centrifugation and then diluting 100 µl of the supernatant solution in 650 µl of distilled water and 250 µl of cefalexin stock solution (50 mg/100 ml)

Stock solutions of drug and internal standard were prepared daily by dissolving 25 mg of ceftazidime base and 12.5 mg of cefalexin in 10 ml and 25 ml of distilled water, respectively.

Apparatus and Chromatographic conditions

The HPLC system consisted of a Waters Associates, Inc model 510 pump and a Water model 481 variable wavelength UV detector set at 257 nm in serum assay and 288 nm in urine assay. A 3.9 mm by 15 cm NOVA pak-C18 stainless steel column (Waters Associates, Inc.) preceeded by a guard column filled with bondapak C18/Coralis 37-50 µm particles were used to achieve separation. The absorbance detector output was monitored with a Waters model 740 data module. The mobile phase was delivered at a flow rate of 1.4 ml/min for both serum and urine analysis. The mobile phase for analysis of serum and urine samples was the mixture of 0.05 M
ammonium dihydrogen phosphate, acetonitrile 7.2% and formic acid 0.0052%, which was filtered and degassed by passing through a 0.45 μm Millipore filter in an all-glass filter apparatus.

Pharmacokinetic analysis

The pharmacokinetic parameters obtained from samples of each subjects were analysed in the following manner:

1. Elimination phase constant (Kel) was the slope of the elimination phase of the pharmacokinetic profile curve plotted between log concentration of serum level and time.

2. Elimination half life (T1/2 β) was calculated by the following equation:

\[ T_{1/2} \beta = \frac{0.693}{K_{el}} \]

3. AUC (area under the curve), Vd (Volume of distribution) and CL (Clearance) were calculated as follows:

\[ \text{AUC} = \frac{A}{\alpha} + \frac{B}{\beta} \]

\[ \text{Vd} = \frac{Dose}{\beta} \text{[AUC]} \]

\[ \text{Cl} = \frac{Dose}{\text{AUC}} \]

A was the intercept on Y axis of the distribution phase. Alpha was the slope of the distribution phase. B was the intercept on Y axis of the elimination phase. Beta was the slope of the elimination phase.

Pharmacokinetic parameters obtained from urine samples of each subjects were established by log amount of ceftazidime in urine divided by time-mid time curve. Elimination phase constant (Kel) was analysed from the slope of these curves and T1/2 β was calculated by the equation above.

Statistical comparisons of T1/2 β (elimination half life) calculated from serum and urine analyses were made by unpaired T test; two tailed tests were applied.

Result:

Table 1. General characteristics of the study volunteers.

<table>
<thead>
<tr>
<th>Age</th>
<th>Weight (kg)</th>
<th>B.P.</th>
<th>Hb</th>
<th>WBC</th>
<th>FBS</th>
<th>BUN</th>
<th>Cr</th>
<th>SGOT</th>
<th>SGPT</th>
<th>AP</th>
<th>U/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>26-41</td>
<td>41-64</td>
<td>100-110</td>
<td>60-80</td>
<td>11.9-14.8</td>
<td>5,000-11,000</td>
<td>18-110</td>
<td>6-14</td>
<td>0.2-0.8</td>
<td>12-32</td>
<td>3-37</td>
<td>12-5-17.5</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>31.8±7.4</td>
<td>50.6±8.3</td>
<td>-</td>
<td>13.4±1.0</td>
<td>80.5±1744.4</td>
<td>97.3±10.9</td>
<td>10.1±2.5</td>
<td>0.53±0.18</td>
<td>23.5±6.4</td>
<td>21.1±6.5</td>
<td>18.9±9.9</td>
</tr>
</tbody>
</table>

Table I shows the general characteristics of all female volunteers. The mean age was 31.8 years. The mean body weight was 50.6 kilograms. The laboratory investigations including complete blood count, fasting blood sugar, kidney and liver function test, urine analysis were all within normal limits.

![Graph showing standard ceftazidime concentration in serum (μg/ml).](image)

Figure 1. Standard ceftazidime concentration in serum (μg/ml).
Figure 2. Standard cefazidime concentration in urine (mg/ml).

Linearity of the detector response was established by preparing a standard curve from an aqueous cefazidime solution in serum and in urine as shown in figure 1 and figure 2. The resultant plots were linear and correlation coefficient ($r^2$) were 0.9997 and 0.9957 respectively in serum and urine assay. The precisions represented by % coefficient of variance (%cv), were 7.9%, 4.8% and 2.1% at 0.5 $\mu$g/ml, 12.5 $\mu$g/ml and 50.0 $\mu$g/ml of serum standard solution respectively. The mean recovery of cefazidime from the serum was 96.71% and the sensitivity was 0.22 $\mu$g/ml. It took about 5 minutes to assay the level of cefazidime by HPLC technique.

HPLC (method) was used for analysis of the female volunteer serum and urine samples. These samples were obtained from volunteers after a 3 minutes intravenous injection of cefazidime. Fig. 3 shows the curve of overall cefazidime concentration in serum versus time. Fig. 4 shows the profile curve of cefazidime concentration in urine. The data from serum assay were fitted to a two-compartment open model. These incuced the distribution phase and elimination phase. We could get the slope from the graph in the elimination phase. It presented as the elimination phase constant (kel) and from this kel value, we could calculate the elimination half life, and extrapolate the curve in the elimination phase to Y axis, to obtain the other parameters (Vd, AUC, clearance). As shown in the table 2, and table 3.

Figure 3. Curve of overall cefazidime concentration in serum versus time. Each point represents the mean of determinations on eight patients. The standard error of mean at point never exceed 16%.
Figure 4. Curve of overall amount of ceftazidime in urine per hour versus time. Each point represents the mean of determinations on six patients. The standard error of mean at each point never exceed 18%.

Table 2. Pharmacokinetic parameters obtained from linear regression analysis of profile curve in serum of each subjects.

<table>
<thead>
<tr>
<th>N=8</th>
<th>Kel(min⁻¹)</th>
<th>T½ β(hr)</th>
<th>AUC (mg.h.Liter⁻¹)</th>
<th>Vd (L)</th>
<th>Cl (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.0093</td>
<td>1.26</td>
<td>170.21</td>
<td>8.27</td>
<td>75.59</td>
</tr>
<tr>
<td>SD</td>
<td>0.014</td>
<td>0.19</td>
<td>24.06</td>
<td>1.73</td>
<td>11.70</td>
</tr>
<tr>
<td>range</td>
<td>0.0076-0.0112</td>
<td>1.03-1.52</td>
<td>128.94-200.77</td>
<td>6.10-10.37</td>
<td>62.87-97.89</td>
</tr>
</tbody>
</table>

Abbreviation : kel, elimination phase constant; t1/2 Beta, eliminated half life; AUC, area under the curve; Vd, volume of distribution ; Cl, Clearance

Table 3. Pharmacokinetic parameters obtained from linear regression analysis of profile curve in urine of each subject.

<table>
<thead>
<tr>
<th>N = 6</th>
<th>Kel (min⁻¹)</th>
<th>T½ β (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0.0083</td>
<td>1.40</td>
</tr>
<tr>
<td>SD</td>
<td>0.0010</td>
<td>0.17</td>
</tr>
<tr>
<td>range</td>
<td>0.0070-0.0096</td>
<td>1.20-1.65</td>
</tr>
</tbody>
</table>
Pharmacokinetic parameters both in serum and in urine are presented in Table 2 and Table 3. It can be seen from this study that the average serum half life and urine half life were 1.26 hr. and 1.40 hr respectively, with the elimination phase constant (kel) of 0.0093 min⁻¹ for serum and 0.0083 for urine samples. For serum assay, the area under the curve (AUC) was 170.21 mg.h. liter. The volume of distribution (Vd) and serum clearance were 8.27 L and 75.59 ml/min respectively.

Table 4 shows the half life in serum and urine in each volunteer. The values were compared by using unpaired t-test, and there was no statistically significant difference.

Table 4. Half life in serum and in urine in each volunteer.

<table>
<thead>
<tr>
<th>Subject No</th>
<th>T1/2 (Serum) (hr)</th>
<th>T1/2 (urine) (hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.27</td>
<td>1.50</td>
</tr>
<tr>
<td>2</td>
<td>1.08</td>
<td>1.39</td>
</tr>
<tr>
<td>3</td>
<td>1.03</td>
<td>1.20</td>
</tr>
<tr>
<td>4</td>
<td>1.44</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>1.41</td>
<td>1.44</td>
</tr>
<tr>
<td>6</td>
<td>1.52</td>
<td>1.65</td>
</tr>
<tr>
<td>7</td>
<td>1.28</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>1.07</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Mean: 1.26; SD: 0.19; N: 8

(Unpaired T-test, N.S.)

Discussion

The HPLC assays presented is a rapid, sensitive and reproducible method for the detection of ceftazidime concentration in both serum and urine. These results from Thai female volunteers were similar with other studies (2, 3) regarding the serum half life, and clearance. From our study we found that the half life from the serum and urine were not statistically significant different, (p < 0.05, unpaired t-Test) as show in table 4. As far we know, the ceftazidime was not metabolized (7). It was completely excreted by the kidneys in unchanged form. We suggest the use of this HPLC method for the determination of ceftazidime pharmacokinetics in urine instead of blood. Since it is easy to collect urine samples, and no blood is needed. In our opinion, the urine assay by HPLC method is also suitable for ceftazidime pharmacokinetic studies in humans.

Acknowledgement

This study was supported by the Rachadapiskompoj China Medical Board Research Funds. We wish to thank Assoc. Prof. Dr. Panpit Suwangoool, Infectious unit, Dept. of Medicine, Faculty of Medicine, Chulalongkorn University for her help in this study, and for the drug supplied (Ceftazidime). We also wish to thank Assoc. Prof. Dr. Ruangchit Panomwanayaudthya, Faculty of Pharmaceutical science, Chulalongkorn University, for her assistance in Pharmacokinetic consultation and also thank Assoc. Prof. Dr. Sompong Limongsanukul and Dr. Nopaoorn Pavijitr for their help in recruiting the volunteers.
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