Effect of Experimental Renal Failure on the Pharmacodynamics of Cefoselis-Induced Seizures in Rats

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We investigated the effect of infusion rate and experimental renal failure on the pharmacodynamics of cefoselis (CFSL)-induced seizures. As an animal model of CFSL-induced seizures, male Wistar rats received an intravenous infusion of CFSL at one of three different rates (1.4—5.8 g/h/rat) until the onset of maximal seizures (which occurred after 8.0 to 36.0 min of infusion). Samples of cerebrospinal fluid (CSF), blood (for serum), and brain were obtained immediately after stopping infusion of CFSL. The serum concentration of CFSL at the onset of seizures increased with increasing infusion rate, but brain and CSF concentrations of CFSL at the onset of seizures were not affected by the infusion rate. Ureter-ligated (UL) and control rats received an intravenous infusion of CFSL at 1.4 g/h/rat until the onset of seizures. Then the same procedure as used to determine the effect of infusion rate on the concentrations of CFSL was carried out. Renal failure was associated with a significant decrease in the amount of CFSL required to induce seizures. Serum, brain, and CSF concentrations of CFSL in UL rats were significantly lower than those in control rats. These results indicate that the experimental strategy and animal model in this investigation would be useful to assess the effects of diseases and other variables on the pharmacodynamics of CFSL-induced seizures and that renal failure is one of the risk factors for neurotoxicity of CFSL.

Key words cefoselis; renal failure; seizure; pharmacodynamics

Cefoselis (CFSL), a fourth-generation parenteral cephalosporin, has been marketed in Japan since September 1998. In December 1998, warnings of central nervous system (CNS) adverse effects such as seizures and confusional states were added to the labeling of CFSL. In many cases, these adverse effects were observed in patients who were elderly and/or had renal failure. Therefore renal failure is considered as one of the risk factors for neurotoxicity of CFSL. Since CFSL is mainly eliminated by renal excretion, renal failure would be associated with increased serum concentrations of CFSL. On the other hand, it is still unknown whether renal failure can alter the pharmacodynamics of CFSL-induced seizures.

Drug administration can be divided into two phases: a pharmacokinetic phase in which dose, dosage form, frequency, and route of administration are related to drug concentration—time relationships in the body; and a pharmacodynamic phase in which the concentration of drug at the site(s) of action is related to the magnitude of the effect(s) produced. The pathophysiologic status of patients can affect both the pharmacokinetics and the pharmacodynamics of a drug. Our knowledge of the effects of disease on the pharmacokinetics of a drug cannot be used to optimize the drug dosage of a patient without information on disease effects on the pharmacodynamics. Therefore it is important to distinguish the effects of disease on the pharmacokinetics from those on the pharmacodynamics. Danhof and Levy reported an experimental strategy to assess the effects of disease and other variables on the pharmacodynamics of drugs with CNS activity. The effects of experimental renal failure on the pharmacodynamics of CNS stimulant drugs such as theophylline, pentylentetrazole, and cimetidine were investigated previously.

There have been clinical case reports of seizures caused by various β-lactam antibiotics such as penicillin, cefazoline, ampicillin, and imipenem. However, to our knowledge, there have been no reports that clarified the effect of renal failure on the pharmacodynamics of β-lactam antibiotic-induced seizures. To assure safe and effective antibiotic drug therapy, it must be determined if renal failure can change the relationship between the concentration and intensity of pharmacologic activity of drugs.

The present study was designed to determine: 1) the suitability of the rat as an animal model of CFSL-induced seizures; 2) the sampling site where CFSL concentrations reflect the drug concentration at the site of neurotoxic action; and 3) the effect of renal failure on the pharmacodynamics of CFSL-induced seizures.

MATERIALS AND METHODS

Animals Male Wistar rats weighing 240 to 300 g were used in this investigation. The rats had an indwelling cannula implanted in the left juglar vein under light ether anesthesia one day before the experiment and they were fasted until the CFSL infusion began.

Chemicals CFSL sulfate (Wincef® for infusion) used for animal experiments was obtained from Fujisawa Pharmaceutical Co., Ltd. Cefpirome sulfate (Broact® injection) used as an internal standard was obtained from Shionogi Co., Ltd. All other chemicals were purchased from commercial sources and used without further purification.

Effect of Infusion Rate on the Concentrations of CFSL in Serum, Brain, and CSF at Onset of Maximal Seizures

CFSL was infused through the cannula at one of three different rates (1.4, 2.9, or 5.8 g/h) in rats. The infusion was stopped immediately at the onset of maximal seizures. The rats were then lightly anesthetized with ether, and samples of CSF, blood, and brain were obtained, in that order. CSF was obtained by cisternal puncture. Blood was obtained from the abdominal aorta and centrifuged to obtain serum. The whole brain was removed and the right half of the cerebrum was used for drug assay.

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**Effect of Renal Failure on the Concentrations of CFSL in Serum, Brain, and CSF at Onset of Maximal Seizures**

Renal failure was produced by bilateral ligation of the ureters (two tight ligatures around each ureter and the ureters cut between the ligatures) about 24 h before the experiment. Sham-operated animals served as controls. Blood samples to determine the concentration of urea nitrogen in serum were drawn from the tail vein just before CFSL infusion. CFSL was infused through the cannula at 1.4 g/h in ureter-ligated (UL) and control rats. Then the same procedure as used to determine the effect of infusion rate on the concentrations of CFSL was carried out. The concentration of urea nitrogen in serum was measured using the Urea Nitrogen B-Test Wako (Wako, Japan). Protein concentration in CSF was determined with the Bio-Rad Protein Assay (Nippon Bio-Rad Laboratories, Japan), using bovine albumin as the standard.

**Assay of CFSL Concentration**

CFSL concentration was determined by HPLC. For the determination of CFSL in serum, serum 100 μl, 0.1 M sodium and potassium–phosphate buffer (pH 7.0) 10 μl containing cephirome sulfate 100 mg/ml as an internal standard and acetoniitrile 100 μl were mixed and vortexed for 10 s, and then centrifuged at 13000 rpm for 2 min. Supernatant 100 μl, and 0.02 M sodium and potassium–phosphate buffer (pH 2.5) 400 μl were mixed and vortexed for 10 s, and 5 μl of the mixture was injected onto the HPLC column.

For the assay of CSF 30 μl, the concentration of the internal standard was reduced to 1 mg/ml and the injection volume was 30 μl.

For the determination of CFSL in brain, the right hemisphere was weighed accurately and homogenized with saline (five-fold volume of the hemisphere weight). The homogenate 200 μl, 0.1 M sodium and potassium–phosphate buffer (pH 7.0) 20 μl containing cefpirome sulfate 1 mg/ml as an internal standard, and acetoniitrile 200 μl were mixed and vortexed for 10 s. Fifty microliters of the final mixture was injected onto the HPLC column.

The HPLC apparatus was an LC-9A (Shimadzu, Japan) equipped with an SPD-6A spectrophotometer (Shimadzu) set at 254 nm. The column was TSKgel ODS-80TM (5 μm, 4.6 mm I.D.×15 cm, TOSOH, Japan). The mobile phase was 0.065% v/v acetoniitrile in 0.02 M sodium and potassium–phosphate buffer (pH 2.5) and the flow rate was 1.0 ml/min.

**Data Analysis** All results were expressed as mean±S.D. Differences in the sample means between UL and control rats were evaluated using the F-test for equality of variances, followed by Student’s t-test or Welch’s t-test. Other experimental results were analyzed by the Tukey–Kramer test.

**RESULTS**

**Effect of Infusion Rate on the Concentrations of CFSL in Serum, Brain, and CSF at Onset of Maximal Seizures**

The results are shown in Table 1. Intravenous infusion of CFSL at rates ranging from 1.4 to 5.8 g/h/rat produced a determinable onset of maximal seizures which occurred at between 8.0±0.7 and 36.0±5.1 min, depending on the drug infusion rate. The total amount of drug administered to the animals ranged from 2.9±0.3 to 3.4±0.5 g/kg and decreased with increasing infusion rate. The concentration of CFSL in serum at the onset of maximal seizures was significantly affected by the rate of drug infusion; the concentration tended to increase with increasing infusion rate. In contrast, CFSL concentrations in the brain and CSF at the onset of maximal seizures were independent of the infusion rate.

**Effect of Renal Failure on the Concentrations of CFSL in Serum, Brain, and CSF at Onset of Maximal Seizures**

The pathophysiological characteristics of both UL and control rats are shown in Table 2. Renal failure significantly increased body weight and serum urea nitrogen levels in rats. The concentration of total protein in CSF tended to be elevated in UL rats, but was not statistically significant compared with control rats.

In a preliminary study, a UL rat received an intravenous infusion of saline at the rate of 12 ml/h for 1 h. No changes in animal behavior were found during saline infusion.

The total dose and concentration of CFSL in serum, brain, and CSF at the onset of maximal seizures are summarized in Table 3. Renal failure was associated with a significant decrease in the amount of CFSL required to induce seizures. Serum, brain, and CSF concentrations of CFSL in UL rats were significantly lower than those in control rats.

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**Table 1. Effect of Infusion Rate on the Concentrations of CFSL in Serum, Brain, and CSF at Onset of Maximal Seizures**

<table>
<thead>
<tr>
<th>Infusion rate (g/h/rat)</th>
<th>1.4</th>
<th>2.9</th>
<th>5.8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>258±19</td>
<td>266±12</td>
<td>264±27</td>
</tr>
<tr>
<td>Total dose (g/kg)</td>
<td>3.4±0.5</td>
<td>3.0±0.3</td>
<td>2.9±0.5*</td>
</tr>
<tr>
<td>Serum concentration (mg/ml)</td>
<td>17.7±1.4</td>
<td>19.5±2.5</td>
<td>21.8±4.0*</td>
</tr>
<tr>
<td>Brain concentration (μg/g)</td>
<td>235±51</td>
<td>247±91</td>
<td>244±46</td>
</tr>
<tr>
<td>CSF concentration (μg/ml)</td>
<td>46.9±19.0</td>
<td>45.5±36.6</td>
<td>36.9±24.0</td>
</tr>
</tbody>
</table>

* Significantly different from results obtained at the lowest infusion rate (p<0.05).

**Table 2. Description of Male Wistar Rats with Bilaterally Ligated Ureters and Their Controls**

<table>
<thead>
<tr>
<th>Control</th>
<th>Renal failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
<td>15</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>259±14</td>
</tr>
<tr>
<td>Serum urea nitrogen (mg/dl)</td>
<td>17±6 (7)</td>
</tr>
<tr>
<td>CSF total protein (mg/dl)</td>
<td>9.6±2.8 (11)</td>
</tr>
</tbody>
</table>

a) Number of animals is in parentheses. *** Significantly different from corresponding control group (p<0.001).

**Table 3. Effect of Renal Failure on the Concentrations of CFSL in Serum, Brain, and CSF at Onset of Maximal Seizures**

<table>
<thead>
<tr>
<th>Infusion rate (min)</th>
<th>Control</th>
<th>Renal failure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total dose (g/kg)</td>
<td>3.63±0.31</td>
<td>1.14±0.36***</td>
</tr>
<tr>
<td>Serum concentration (mg/ml)</td>
<td>18.3±2.1</td>
<td>3.8±1.3***</td>
</tr>
<tr>
<td>Brain concentration (μg/g)</td>
<td>260±56</td>
<td>42±16***</td>
</tr>
<tr>
<td>CSF concentration (μg/ml)</td>
<td>63.7±11.3</td>
<td>31.6±24.9*</td>
</tr>
</tbody>
</table>

* Significantly different from corresponding control group (p<0.05). *** Significantly different from corresponding control group (p<0.001).
DISCUSSION

Drugs that act directly and reversibly, have no pharmacologically active metabolites, and are not subject to the development of acute tolerance may be expected to produce their apparent pharmacologic effect(s) when their concentration(s) at the site of action reach and exceed the minimum effective concentration. We need to determine the sampling site that reflects drug concentrations in the site of action because it is technically impossible to measure drug concentrations in the immediate environment of the site of action. For this purpose, the drug is administered by constant-rate intravenous infusion at different rates until the onset of a suitable pharmacologic endpoint and drug concentration in various fluids and tissues (serum, brain, and CSF) at that time are determined. Sites at which the drug concentration at the onset of action is dependent on infusion rate (the concentration tends to increase with increasing infusion rate) can be considered to be in slow equilibrium with the site of action. Any fluid or tissue that yields the same drug concentration at onset of action, independent of the rate of drug administration, must be considered as being in “instantaneous” distribution equilibrium with the site of action.13

The results of this study show that CFSL concentration in the serum at the onset of maximal seizures is significantly affected by the rate of drug infusion, whereas CFSL concentrations in the brain and CSF at the pharmacologic endpoint are independent of infusion rate. These results suggest that CFSL equilibrates slowly between serum and the site of action, and drug concentrations in the brain and CSF are more appropriate indices of the drug concentration in the site of action.

We also investigated the effect of experimental renal failure on the pharmacodynamics of CFSL-induced seizures in rats. In the present study, renal failure was associated with a significant decrease in the amount of CFSL required to induce seizures. This result suggests that renal failure is one of the risk factors for CFSL-induced seizures, and this is consistent with recent clinical findings of a higher incidence of CFSL-induced neurotoxicity in patients with renal failure.17 In addition, serum, brain, and CSF concentrations of CFSL in UL rats were significantly lower than those in control rats. This indicates that renal failure is associated with changes in the pharmacodynamics of CFSL-induced seizures.

Renal failure could change the pharmacokinetics of CFSL because CFSL is mainly eliminated by renal excretion.3 The permeability of the blood-CSF barrier may be increased as reflected by increased protein concentration in the CSF. If renal failure affected only the pharmacokinetics of CFSL and/or the permeability of CFSL at the blood-CSF barrier, there would be no differences in the CFSL concentration in the brain and CSF at the onset of seizures between control and UL rats. In this study, however, UL rats had lower brain and CSF concentrations of CFSL at the onset of seizures compared with the corresponding results in control rats. This indicates that the cause of the change in drug effect with renal failure is not only the delay in CFSL elimination and the increased permeability of CFSL at the blood-CSF barrier but also the increased CNS sensitivity to CFSL-induced seizures.

The effects of experimental renal failure on the pharmacodynamics of other CNS-active drugs were investigated previously.4—6,12,13 Ramzan and Levy3 reported that the theophylline concentrations in the serum, brain, and CSF at the onset of maximal seizures in rats with renal failure by ureter ligation were lower than those in control rats. Hoffman and Levy14 reported that the potentiation of the convulsive effect of theophylline in rats with renal failure was counteracted by oral administration of activated charcoal that presumably interrupts the cycling of the endogenous substance(s) between blood and the gastrointestinal tract. These indicate that changes in drug effects under renal impairment may be due to, at least in part, an increase in levels of one or more endogenous substance(s) that alter the pharmacological effects of certain drugs in uremic rats.

The uremic blood apparently contains elevated concentrations of one or more endogenous substances, the so-called uremic toxins, and these retained substances probably play a major role as toxins in the pathogenesis of uremia, either working singly or in combination.5,15 Guanidino compounds are believed to be toxic agents in patients with renal insufficiency. Gamma-guanidinobutyric acid was shown to induce tonic and clonic seizures following intracisternal injection in rabbits.16 De Deyn et al. reported that guanidino compounds were found to be increased in the serum and CSF of uremic patients17 and guanidinosuccinic acid induced behavioral convulsions after intraperitoneal injection in mice.18 In addition, four guanidino compounds (guanidinosuccinic acid, creatinine, guanidine, and methylguanidine) were shown to inhibit gamma-aminobutyric acid and glycine responses on mouse neurons in cell culture.19 These suggest that guanidino compounds can contribute to increased neurotoxic action of certain drugs in uremia.

We used UL rats as a model of renal failure in this study. Ramzan and Levy3 reported that the effect of impaired renal function on the convulsant activity of pentylentetrazol in UL rats was different from that in uranyl nitrate-treated rats. Further investigation using more than one experimental model of disease is thus warranted.

In conclusion, renal failure is associated with increased CNS sensitivity to CFSL-induced seizures, and the experimental strategy and animal model in this investigation would be useful to assess the effects of disease and other variables on the pharmacodynamics of CFSL-induced seizures.

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REFERENCES