Effect of Acute Renal Failure on Neurotoxicity of Cimetidine in Rats

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Purpose. We investigated the effect of acute renal failure on the neurotoxicity of cimetidine in rats.

Methods. Experimental acute renal failure was produced by bilateral ureteral ligation. Cimetidine was intravenously infused to ureter-ligated (UL) and control rats, and cimetidine concentration in plasma, brain and cerebrospinal fluid (CSF) were compared.

Results. The cimetidine concentration in plasma was rapidly increased in UL rats as compared to control rats. The cimetidine concentration in CSF at the onset of convulsion did not depend on the infusion rate, suggesting that cimetidine in CSF equilibrates rapidly with the site of action for clonic convulsion. The cimetidine concentration in CSF of UL rats at the onset of clonic convulsion was lower than those of control rats.

Conclusions. Increased sensitivity to the drug on the central nervous system may contribute to increased toxicity of cimetidine with renal failure.

KEY WORDS: cimetidine; acute renal failure; clonic convulsion; neurotoxicity.

INTRODUCTION

There have been many clinical case reports of side effects on the central nervous system (CNS) brought on by histamine H2 receptor antagonists. These effects include delirium, mental confusion and convulsion in severe cases (1–4).

Recently, we reported that renal dysfunction is a risk factor for ranitidine neurotoxicity, resulting from elevation in plasma and brain concentration of the drug as a result of impaired renal function in mice (5). Further, we suggested that renal and/or hepatic disease are risk factors for neurotoxicity based on the retrospective analysis of pharmacokinetic and toxicodynamic data of H2 antagonists in patients (6). The CNS disturbance may be caused by the blockade of the histamine H2 receptor in the brain and the intrinsic toxicity of H2 antagonists to CNS does not change in renal and/or hepatic disease. However, they are not confirmed. In this study, cimetidine was intravenously infused to ureter ligated (UL) and control rats to determine the cimetidine concentration in plasma, cerebrospinal fluid (CSF) and brain at the onset of clonic convulsion. Then, the effect of acute renal failure on intrinsic neurotoxicity and the distribution kinetics of cimetidine to CNS were investigated.

MATERIALS AND METHODS

Animals

Male Wistar rats (Nippon Igakaku Dobutsu) were housed in a cage maintained at 22 ± 2°C for 7 days with free access to water and the cube diet (MF; Oriented Yeast Co.), and animals weighing 220–280 g were used in all experiments.

Chemicals

Cimetidine (Tagamet® Injection) used for animal experiments was purchased from Smith-Klein-Beecham (U.K.). Cimetidine and ranitidine used as standards for quantitation were generously supplied by Smith-Klein-Beecham and Glaxo Pharmaceuticals Japan. All other chemicals were purchased from commercial sources and used without further purification.

Preparation of Rats with Experimental Renal Failure

Renal failure was produced by bilateral ligation of ureters (two tight ligatures around each ureter and the ureters cut between the ligatures) about 48 hr before the cimetidine infusion (7,8). The rats had an indwelling cannula (PE-10, Becton Dickinson, U.S.A.) implanted in the right jugular vein during surgery for ureter ligation. Sham-operated animals served as control rats. The concentration of urea nitrogen, glutamic oxaloacetic transaminase (GOT) activity and glutamic pyruvic transaminase (GPT) activity in plasma were measured by Seralyzer Dry Chemistry System (Miles Inc., U.S.A.), GOT UV-Test Wako (Wako, Japan) and GPT UV-Test Wako (Wako), respectively. Albumin concentration in plasma and total protein concentration in CSF were assayed by Albumin Test Wako (Wako) and Micro TP Test Wako (Wako), respectively. The animals with plasma urea nitrogen of more than 100 mg/dL, and GOT and GPT of less than 50 and 20 IU/L, respectively, were supplied as the rats with acute renal failure.

Effect of Acute Renal Failure on Plasma Concentration of Cimetidine

Under ether anesthesia, the right femoral artery and vein were cannulated for blood sampling and drug administration, respectively. The animal was placed to a dorsal position and the body temperature was maintained at 37.5°C by isothermal pad. After complete recovery from anesthesia, cimetidine injection was infused with an infusion pump at the constant rate of 6.5 mg/min. Blood samples were withdrawn at 1, 2, 3, 5, 10, 15, 20, 25 and 30 min after the start of infusion. If the animal died within 30 min, the last sample was obtained at the death.

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Effect of Acute Renal Failure on Concentration of Cimetidine in Plasma, CSF and Brain at the Onset of Convulsion

The polyethylene tube of 50 cm length was attached to the indwelling cannula of right jugular vein, and the other end of the catheter was attached to a syringe. Then the animal was transferred to the clear plastic box and left for 10 min. Rats were freely moving during the experiment. Cimetidine was infused at 4 different rates of 1.625, 3.25, 6.5 and 13.0 mg/ml to UL rats and at 3 different rates of 3.25, 6.5 and 13.0 mg/ml to control rats. The infusion was stopped at the onset of clonic convulsions, and the rats were lightly anesthetized with ether at this time. Clonic movement of the limbs lasting more than 4 sec scored as clonic convulsions. CSF was obtained by cisternal puncture (8.9) and the blood was drawn from abdominal aorta and centrifuged immediately to obtain plasma. Whole brain was taken and the dexter half of the cerebrum was used for drug assay. All samples were stored at −80°C until analysis.

Assay of Cimetidine Concentration

The cimetidine concentration was determined by HPLC method. For the determination of cimetidine in plasma, 25 μl of plasma, 100 μl of 100 μg/ml ranitidine solution as an internal standard, 100 μl of 5 M NaOH and 5 ml of CH2Cl2 were mixed and shaken for 10 min., then centrifuged at 1650 g for 10 min. After the upper aqueous phase was removed, 4 ml of the organic phase were transferred to another tube and evaporated. The residue was dissolved with 100 μl of mobile phase and 25 μl was subjected to HPLC. Plasma unbound fraction was determined by ultrafiltration (MPS-3 centrifree, Amicon, U.S.A.) and 25 μl of filtrate was subjected to the same procedure as described above to determine unbound concentration of cimetidine in plasma (Cpf). For determination of cimetidine concentration in blood, 50 μl of water was added to 25 μl of blood to cause hemolysis. Then the same procedure as used to measure plasma concentration was carried out.

Brain tissue with 100 μl of 50 μg/ml ranitidine solution and 1 ml of saline was homogenized for 1 min on ice. Then, 100 μl of 1 M NaOH was added to the homogenate and extracted with 5 ml of CH2Cl2. After the upper aqueous phase was removed, 3 ml of the organic phase were transferred to another tube and evaporated. The residue was dissolved with 100 μl of mobile phase, then centrifuged at 10000 g. Twenty-five μl were subjected to HPLC. The measured concentration of cimetidine in the brain was corrected for remaining blood as described previously (5). Briefly, 131I-human serum albumin was purified by Sephadex G-25 medium gel chromatography, and 324 kBq/kg was intravenously administered to UL and control rats and the radioactivities in blood and brain at 5 min were counted. The brain capillary volume (ml/g brain) was calculated as the ratio of radioactivities in the brain to those in blood. Then, true brain concentration was estimated by correcting for the drug amount in remaining blood in the brain. For the determination of cimetidine in CSF (C_{CSF}), CSF sample was directly injected to the HPLC and the cimetidine concentration was determined by absolute calibration method.

The HPLC apparatus was an LC-6A (Shimadzu, Japan) equipped with an SPD-6A spectrophotometer (Shimadzu) set at 225 nm. The column was 4 × 250 mm stainless tube packed with the Senshu gel 7C18 (Senshu, Japan). The mobile phase was of 5 mM NaH2PO4 and 5 mM tetramethylammonium chloride in 5% CH3CN and pumped at the rate of 2 ml/min. The column temperature was maintained at 40°C. The detection limits of cimetidine in plasma, blood, CSF and brain were 1 μg/ml, 1 μg/ml, 50 ng/ml and 500 ng/g, respectively.

Data Analysis

Difference of the sample means between UL and control rats were evaluated by Welch’s test. Other experimental results were analyzed by one-way ANOVA; when differences were noted, the means were compared by the Tukey-Kramer test.

RESULTS

Pathophysiological characteristics of both UL and control rats were shown in Table I. Plasma urea nitrogen was elevated substantially, while GOT and GPT activity in plasma was normal in UL rats. Total protein concentration in CSF significantly increased in UL rats.

Profiles of cimetidine concentration in the plasma in UL and control rats during 6.5 mg/min infusion are shown in Fig. 1. In UL rats, plasma concentration of cimetidine increased as compared to control rats. The increase of plasma unbound fraction of cimetidine in UL rats was not significant (Table II). The total dose and concentration of cimetidine in plasma, CSF and brain at the onset of convulsion are summarized in Table II. In the control rats, the onset time for clonic convulsion was shortened as the infusion rate increased, and cimetidine concentration in plasma at the onset during 13.0 mg/min infusion was higher than those of other infusion rates, while cimetidine concentrations in CSF and the brain at the onset of clonic convulsion were not infusion rate dependent. On the other hand, cimetidine concentrations in CSF at the onset of clonic convulsion was not infusion rate dependent in UL rats, while brain concentration increased as the infusion rate increased.

In UL rats, onset time was significantly shorter than those of control rats during all infusion rates studied. Further investigation was done using additional samples.

### Table I. Characteristics of the Wistar Rats Used in this Investigation

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Renal Failure</th>
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<tbody>
<tr>
<td>Body weight (g)</td>
<td>239 ± 3</td>
<td>238 ± 4</td>
</tr>
<tr>
<td>Plasma urea nitrogen (mg/dl)</td>
<td>23.7 ± 1.2</td>
<td>296 ± 9***</td>
</tr>
<tr>
<td>Plasma GPT (I.U./l)</td>
<td>47.4 ± 2.1</td>
<td>41.4 ± 1.5*</td>
</tr>
<tr>
<td>Plasma GOT (I.U./l)</td>
<td>12.9 ± 1.0</td>
<td>11.8 ± 1.2</td>
</tr>
<tr>
<td>Plasma albumin (g/dl)</td>
<td>3.94 ± 0.15</td>
<td>3.53 ± 0.19</td>
</tr>
<tr>
<td>CSF total protein (g/dl)</td>
<td>0.021 ± 0.001</td>
<td>0.106 ± 0.020***</td>
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</tbody>
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Results are represented as mean ± S.E. (n = 18)

* Significantly different from control group (p < 0.05, Welch’s test)

*** Significantly different from control group (p < 0.001, Welch’s test)

GOT: Glutamic oxaloacetic transaminase

GPT: Glutamic pyruvic transaminase