Effect of Acute Renal Failure on Neurotoxicity of Cimetidine in Rats

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Received January 12, 1995; accepted July 18, 1995

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 H_2 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 H_2 antagonists in patients (6). The CNS disturbance may be caused by the blockade of the histamine H_2 receptor in the brain and the intrinsic toxicity of H_2 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.

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MATERIALS AND METHODS

Animals

Male Wistar rats (Nippon Ikagaku 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 canulated 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.

0724-8741/95/1200-1953$07.50/0

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 CH_2Cl_2 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 CH_2Cl_2. 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, ^{131}I-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 $7C_{18}H$ (Senshu, Japan). The mobile phase was of 5 mM NaH_2PO_4 and 5 mM tetramethylammonium chloride in 5% CH_3CN 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. Fur-

Table I. Characteristics of the Wistar Rats used in this Investigation

	Control	Renal Failure
Body weight (g)	239 ± 3	238 ± 4
Plasma urea nitrogen (mg/dl)	23.7 ± 1.2	296 ± 9***
Plasma GPT (I.U./l)	47.4 ± 2.1	41.4 ± 1.5*
Plasma GOT (I.U./l)	12.9 ± 1.0	11.8 ± 1.2
Plasma albumin (g/dl)	3.94 ± 0.15	3.53 ± 0.19
CSF total protein (g/dl)	0.021 ± 0.001	0.106 ± 0.020***

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

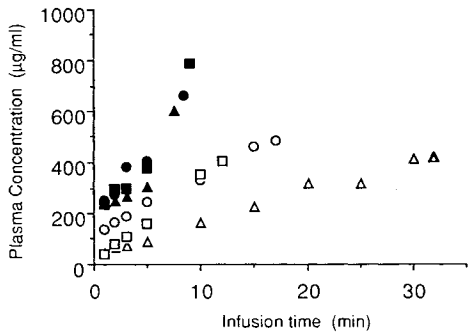

Fig. 1. Effect of acute renal failure on plasma concentration-time profiles of cimetidine during constant infusion. Infusion rate was set at 6.5 mg/min. Each symbol represents the data from one rat; open symbols (rat 1: △, rat 2: □, rat 3: ○) represent the data from control rats, closed symbols (rat 4: ▲, rat 5: ■, rat 6: ●) represent the data from rats with acute renal failure.

ther, cimetidine concentration in CSF at the onset of convulsion in UL rats is significantly lower than that of control rats during all infusion rates. Cimetidine concentrations in plasma at the onset of convulsion in UL rats were also lower than those in control rats during 3.25 and 6.5 mg/min infusion.

The relationship between onset time of convulsion and the CSF/plasma unbound concentration ratio were shown in Fig. 2. The C_{CSF}/Cpf values in UL and control rats do not show significant difference in any infusion rates. It may take more than 7 min for the cimetidine to equilibrate between the plasma and the effective site (CSF) responsible for clonic convulsion. C_{CSF}/Cpf values of cimetidine in UL rats were significantly lower than that in control rats after 15 min.

DISCUSSION

We investigated the effect of renal failure on neurotoxic efficacy of cimetidine in rats with experimental acute renal failure induced by bilateral ureter ligation. Since plasma concentration of cimetidine in UL rats was higher than that of control rats during cimetidine infusion at the constant rate of 6.5 mg/min (Fig 1), accumulation of the drug due to the impaired renal function must be considered as the cause of increased toxicity of cimetidine in renal failure (Table II).

It is very important to clarify the concentration-effect relationship of drugs for the evaluation of pharmacological/toxicological efficacy. Though it is ideal to determine the drug concentration at the effective site, we could determine the drug concentration only in tissues or biological fluids. In order to determine the appropriate tissue to reflect the drug concentration at the effective site, other investigators (9,10) administered the drugs to animals at various infusion rate, and determined the drug concentration in plasma, CSF and brain at the pharmacological onset of convulsion. In the present study, cimetidine was infused to UL and control rats at 4 and 3 different rates, respectively, and the drug concentrations in plasma, CSF and brain of these rats were determined. In control rats, total and unbound concentration of cimetidine in plasma at the onset of convulsion depended on infusion rate. Control rats manifested the convulsion at 6.7 min with the highest rate of 13 mg/min, and the total and unbound cimetidine concentration in plasma were significantly higher than those in other infusion rates. Therefore, it may take more than 7 min for the drug concentration to equilibrate between the plasma and the effective site. On the other hand, drug concentrations in CSF and brain at the onset of convulsion were independent to the infusion rate, suggesting that the effect compartment may be the CSF and the brain. In addition, brain concentrations at the onset of convulsion in UL rats changed dose dependently, suggesting that the drug disposition in the brain of UL rats was different from that of control rats, and the CSF concentration is more appropriate index of the drug concentration in effective site.

Change of drug effects under renal impairment may be due to the increase of drug concentration in plasma and the increase of unbound fraction caused by the impaired renal function. In this study, however, unbound concentration of cimetidine in plasma was lower in UL rats than that in control rats (Table II). Cimetidine concentration in CSF was also significantly lower than that in the control rats. Since we believe the CNS effect occurs at a certain concentration of cimetidine, these results suggest that the sensitivity of CNS to cimetidine may be enhanced in UL rats. Cimetidine concentration in brain at the onset of clonic convulsion in UL rats was also lower than that in control rats at the low infusion rate. However, cimetidine concentration in brain at the onset increased infusion-rate dependently. Brain and the effective site may rapidly equilibrate in control rats, but the change of distribution kinetics in UL rats may be considered. Phenobarbital concentration in the brain also did not rapidly equilibrate to the effective site responsible for elimination of righting reflex in rats with renal failure (7).

Effects of experimental renal failure on pharmacological activity by other drugs to CNS were investigated previously. Danhof et al. (7) reported that the phenobarbital concentration in CSF at the onset of loss of righting reflex in rats with renal failure was lower than that of control rats. Similar result was obtained in the theophylline-induced seizure study by Ramzan and Levy (6). Increase of one or more endogenous substance(s) that alter the pharmacological effect of certain drugs in uremic animals may be one of the causes of these pharmacodynamical changes. Hoffman and Levy (11) reported that the potentiation of convulsive effect of theophylline in uremic rats was counteracted by oral administration of activated charcoal that may reduce the concentration of circulating endogenous substance(s). Further, Hisaoka and Levy (12) reported the hypnotic effect of phenobarbital was potentiated by administration of dialysate of plasma obtained from uremic animals. Further investigation will be required to elucidate the mechanism of potentiation of CNS effect of cimetidine in UL rats.

To evaluate the effect of renal failure on the distribution of cimetidine to CNS, C_{CSF}/Cpf at the onset of convulsion was plotted against the onset time during i.v. infusion of cimetidine with various infusion rate (Fig. 2). The slowest infusion rate of 1.625 mg/min was carried out in UL rats to compare the C_{CSF}/Cpf between UL and control rats in a comparable time span. The permeability of cimetidine at the blood-CSF barrier may increase as reflected by increase of protein concentration in CSF (Table I). However, C_{CSF}/Cpf ratio of cimetidine in UL rats was significantly lower than that in the control rats at 15 min or more after the start of infusion, when the plasma compartment may equilibrate to

Table II. Effect of Renal Failure on Concentration of Cimetidine at Onset of Clonic Seizure in Rats

Infusion rate	1.625 mg/min	3.25 mg/min		6.5 mg/min		13.0 mg/min	
	Renal Failure	Control	Renal Failure	Control	Renal Failure	Control	Renal Failure
No. of animals	3	5	5	8	8	5	5
Onset time (min)#	22.0 ± 1.6	27.0 ± 0.8	10.6 ± 1.6***‡	15.1 ± 2.1‡	7.01 ± 0.86**‡	6.68 ± 0.50‡	4.12 ± 0.20**‡
Total dose (mg/kg)	154 ± 10	389 ± 9	151 ± 22***	395 ± 51	183 ± 23**	370 ± 34	232 ± 9*
Plasma concentration							
Total ⟨Cp⟩ (μg/ml)	248 ± 30	368 ± 16	200 ± 37**	355 ± 23	281 ± 25*	506 ± 40†	444 ± 59†
Free ⟨Cp,f⟩ (μg/ml)	214 ± 30	304 ± 21	173 ± 30**	285 ± 19	255 ± 25	407 ± 31†	376 ± 61
Free fraction (%)	85.6 ± 1.7	82.1 ± 2.7	87.5 ± 2.4	80.3 ± 1.9	90.0 ± 2.3**	81.0 ± 3.9	82.6 ± 4.2
CSF conc. (μg/ml)	5.35 ± 0.26	9.91 ± 0.40	3.79 ± 0.75***	11.1 ± 0.7	6.76 ± 0.90***	9.93 ± 1.4	4.70 ± 0.45*
Brain conc. (μg/g)	3.45 ± 0.88	9.49 ± 0.46	4.92 ± 1.13*	9.38 ± 0.92	8.21 ± 1.10	8.04 ± 2.81	11.7 ± 3.0

Results are repesented as mean ± S.E.
* Significantly different from corresponding control group ($p < 0.05$, Welch's test).
** Significantly different from corresponding control group ($p < 0.01$, Welch's test).
*** Significantly different from corresponding control group ($p < 0.001$, Welch's test).
# All results were significantly affected by infusion rate, ($p < 0.001$, one-way analysis of variance).
† Significantly different from corresponding result obtained at the lowest infusion rate, ($p < 0.05$, Tukey-Kramer multiple comparisons test).
‡ Significantly different from corresponding result obtained at the lowest infusion rate ($p < 0.001$, Tukey-Kramer multiple comparisons test).

the effective site. The cause of decrease in CNS distribution of cimetidine in UL rats is still unknown.

The accumulation of active metabolites may be another possibility for the increased sensitivity in UL rats. After i.v. administration of cimetidine, about 70% was excreted in urine as unchanged drug in rats, dogs and human (13). Several metabolites including cimetidine sulfoxide were excreted in urine in these species. These metabolites are pharmacologically inactive, but their toxicological activities are unknown. Therefore, the possibility that these metabolites contribute the increased sensitivity in UL rats can not be clearly excluded.

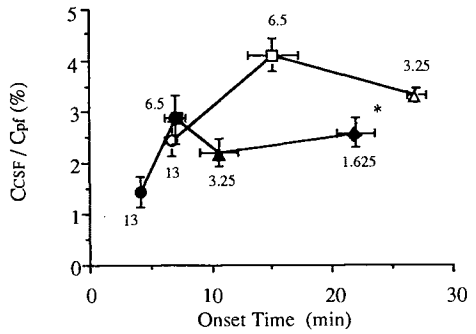

Fig. 2. Effect of acute renal failure on CNS distribution of cimetidine in rats. Infusion rates were at 3.25 (△), 6.5 (□), 13.0 mg/ml (○) for control rats and 1.625 (♦), 3.25 (▲), 6.5 (■) and 13.0 mg/min (●) for rats with acute renal failure. Numbers in the figure represent the infusion rate in mg/min. Results are represented as mean ± S.E. (n = 3–8). $C_{csf}/Cp,f$ values in UL and control rats are not significantly different among all infusion rates. *Significantly different from both control groups at the infusion rate of 3.25 and 6.5 mg/ml.

In conclusion, renal failure may be a serious risk factor for cimetidine-induced neurotoxicity. The accumulation of cimetidine in the body due to the impaired renal function and the enhanced sensitivity of CNS to cimetidine may contribute to increased neurotoxicity. Further investigation required to clarify the mechanism of change in CNS sensitivity and CNS distribution kinetics of cimetidine in rats with renal failure.

ACKNOWLEDGMENTS

This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

REFERENCES

1. J. J. Schentag. Cimetidine-associated mental confusion: Further studies in 36 severely ill patients. *Ther. Drug Monit.*, 2:133–142 (1980).
2. J. J. Schentag, F. B. Cerra, G. Calleri, E. DeGlopper, J. Q. Rose and H. Bernhard. Pharmacokinetic and clinical studies in pa-

tients with cimetidine-associated mental confusion. *Lancet*, 1:177–181 (1979).
3. M. E. Edmonds, R. F. U. Ashford, M. K. Brenner and A. Saunders. Cimetidine:dose neurotoxicity occur? Report of three cases. *J. Roy. Soc. Med.*, 72:172–175 (1979).
4. B. J. Kimerblatt, F. B. Cerra, G. Calleri, M. J. Berg, M. A. Mcmillen and J. J. Schentag. Dose of serum concentration relationships in cimetidine-associated mental confusion. *Gastroenterology*, 78:791–795 (1980).
5. M. Shimokawa, K. Yamamoto, J. Kawakami, Y. Sawada and T. Iga. Effect of renal or hepatic dysfunction on neurotoxic convulsion induced by ranitidine in mice. *Pharm. Res.*, 11:1519–1523 (1994).
6. M. Shimokawa, K. Yamamoto, Y. Sawada and T. Iga. Pharmacokinetic/pharmacodynamic evaluation of the risk of adverse reaction on central nervous system induced by H2 receptor antagonists: Hepatic and/or renal disease as risk factors. *Xenobio. Metab. Dispos.*, 8:295–305 (1993)
7. M. Danhof, M. Hisaoka and G. Levy. Kinetics of drug action in disease states. II. Effect of experimental renal dysfunction on phenobarbital concentrations in rats at onset of loss of righting reflex. *J. Pharmacol. Exp. Ther.*, 230:627–631 (1984).
8. I. M. Ramzan and G. Levy. Kinetics of drug action in disease states. XVIII. Effect of experimental renal failure on pharmacodynamics of theophylline-induced seizures in rats. *J. Pharmacol. Exp. Ther.*, 240:584–588 (1986).
9. I. M. Ramzan and G. Levy. Kinetics of drug action in disease states. XVI. Pharmacodynamics of theophylline-induced seizures in rats. *J. Pharmacol. Exp. Ther.*, 236:708–713 (1986).
10. M. Danhof and G. Levy. Kinetics of Drug Action in Disease States. I. Effect of infusion rate on phenobarbital concentrations in serum, brain and cerebrospinal fluid of normal rats at onset of loss of righting reflex. *J. Pharmacol. Exp. Ther.*, 229:44–50 (1983).
11. A. Hoffman and G. Levy. Kinetics of drug action in disease States. XXXIX. Effect of orally administered activated charcoal on the hypnotic activity of phenobarbital and the neurotoxicity of theophylline administered intravenously to rats with renal failure. *Pharm. Res.*, 7:242–246 (1990).
12. M. Hisaoka and G. Levy. Kinetics of drug action in disease states. XIII. Effect of dialyzable component(s) of uremic blood on phenobarbital concentrations in rats at onset of loss of righting reflex. *J. Pharmacol. Exp. Ther.*, 234:180–183 (1985).
13. D. C. Taylor, P. R. Cresswell and D. C. Bartlett. The metabolism and elimination of cimetidine, a histamine H_2-receptor antagonist, in the rat, dog, and man. Drug Metab. Dispos., 6:21–30 (1978).