Effect of Renal or Hepatic Dysfunction on Neurotoxic Convulsion Induced by Ranitidine in Mice

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We investigated the effect of acute renal and hepatic dysfunction on the neurotoxicity of ranitidine, a histamine H_2 receptor antagonist. Experimental acute hepatic and renal dysfunction in mice were produced by i.p. injection of uranyl nitrate (UN) and carbon tetrachloride (CT), respectively. Ranitidine was then constantly infused into the tail vein until the onset of clonic convulsion. When compared to control mice, UN treated mice had a significantly shorter onset time to clonic convulsion, lower total dose and higher plasma concentration at initiation of clonic convulsion. In contrast, the convulsive threshold concentration in the brain of UN treated mice was not significantly different from that of control mice. In CT treated mice, all pharmacokinetic and pharmacodynamic data described above were not significantly different from those of the control mice. No significant difference in the brain/plasma concentration ratio was observed between both disease models and the corresponding control mice. Finally, the effect of UN and CT treatment on the convulsive potency after intracerebral (i.c.) administration of ranitidine was investigated in mice. Potentiation of the intrinsic neurotoxic sensitivity to ranitidine could not be demonstrated for mice with renal or hepatic dysfunction. From these findings, we conclude that renal dysfunction is a risk factor for ranitidine neurotoxicity, and this increased risk results from increase in the drug concentration in plasma and brain as a result of impaired renal excretion. No apparent effect of acute hepatic dysfunction was observed on both the pharmacokinetic and pharmacodynamic behavior of the drug.

KEY WORDS: ranitidine; neurotoxicity; renal dysfunction; hepatic dysfunction; H_2 antagonists.

INTRODUCTION

There have been many clinical reports concerning the induction of central nervous system (CNS) adverse effects by histamine H_2 receptor antagonists. These adverse effects include delirium, mental confusion and convulsion in severe cases (1–4). Of note, patients with renal and/or hepatic disease experienced these effects despite normal dosing schedule of H_2 antagonists (5). Based on the retrospective analysis of pharmacokinetic and toxicodynamic data of H_2 antagonists in patients, we reported evidence that renal and/or hepatic disease are risk factors for neurotoxicity (6). The elevation of drug level in the plasma and brain due to the impaired urinary excretion of drugs in patients with renal disease and the increased brain uptake of drugs in patients with hepatic disease may have accounted for the high incidence of neurotoxicity. However, this analysis was based on three assumptions: 1) CNS disturbance is caused by the blockade of the histamine H_2 receptor in the brain, 2) there is no difference in the distribution to the CNS of each H_2 antagonist among patients with or without renal/hepatic disease and 3) the intrinsic toxic sensitivity of the CNS to H_2 antagonists does not change in each disease state. In order to investigate the effect of these disease states on neurotoxicity of H_2 antagonists, we used animal models for both renal and hepatic disease.

Recently, we showed that a possible mechanism for the neurotoxic convulsion induced by H_2 antagonists was the blockade of histamine H_2 receptors in the brain, and this process was not associated with gamma-aminobutyric acid or N-methyl D-aspartate mediated neurotransmission (7). In this study, the effect of renal and hepatic disease states on the plasma and brain concentrations of ranitidine and their impact of neurotoxicity to the drug were investigated by using experimental disease models in mice.

MATERIALS AND METHOD

Animals

Male ddY mice (5 weeks of age) weighing 20 g (Nihon 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; Oriental Yeast Co.), and only animals weighing 25–30 g were used in all experiments.

Chemicals

Ranitidine hydrochloride was generously supplied by Glaxo Pharmaceuticals Japan. ^{131}I-Human serum albumin (18.5 MBq) purchased from Daiichi Radio Isotope Laboratories (Japan) was purified by Sephadex G-25 Medium gel chromatographies (Pharmacia, Sweden), and preparations containing less than 0.3% of free iodide by ultrafiltration (MPS3 centrifree, amicon) were used for experiments. All other chemicals were purchased from commercial sources and used without further purification.

Preparation of Mice with Experimental Renal or Hepatic Dysfunction

For the production of mice with experimental renal dysfunction, 20 mg/kg of UN were administered i.p. (8–10). The animals were used for experiments 5 days following injections. For the production of mice with experimental hepatic dysfunction, the mixture of CT and olive oil (1:4, v/v) at the dose of 10 ml/kg were administered i.p. to mice (11). The mice were used for experiments 24 hr after treatment. The concentration of urea nitrogen, aspartate aminotransferase (GOT) activity and alanine aminotransferase (GPT) activity in plasma were measured by Seralyzer Dry Chemistry System (Miles Inc., U.S.A.). The animals with plasma urea nitrogen of more than 100 mg/dl and with both GOT and GPT more than 5000 unit/L were used as the mice with experimental acute renal and hepatic dysfunction, respectively. Table 1 summarizes the pathophysiological changes of UN and CT intoxication.

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Threshold Concentration of Ranitidine in Plasma and the Brain After Manifestation of Clonic Convulsion

Ranitidine was infused to mice at a constant rate of 1.12 μmol/min. The mice were allowed to freely move during the experiment. At the onset of clonic convulsion, the animals were decapitated to collect blood and brain tissue. Blood was centrifuged immediately to obtain plasma. Plasma and the brain tissue samples were stored at −20°C until analysis.

The Incidence of Convulsion After Intracerebral (i.c.) Administration of Ranitidine to Mice

An isotonic ranitidine solution of 66.2 mM, adjusted to pH 6.0 was prepared for i.c. administration. In the preliminary study, 50% of animals manifest the clonic convulsion at this dose. Mice were grasped firmly, and the skin was incised to expose the skull. To the injection site, 3 mm anterior, 2 mm left and 4 mm deeps to the lambdoid suture, 10 μl of drug solution were administered through a 27 G needle. Then the animals were placed in a clear plastic box and were observed for 2 hr for the onset of clonic or tonic convulsion, or death.

Correction for Remaining Blood in the Brain Sample

The apparent concentration of ranitidine in the brain (C_{brain}) was corrected for that concentration remaining in residual blood in the brain tissue sample. In order to do so, we measured the blood/plasma partition coefficient (RB) and the brain capillary volume. To measure RB of ranitidine, the animals were decapitated and ranitidine concentration in blood and plasma were determined at 10, 15, 20, 25 and 30 min after i.v. bolus administration of 157 μmol/kg of drug. All data were averaged, since no time or concentration dependency was observed. At 2 min after i.v. bolus administration of ^{131}I-human serum albumin (925 KBq/kg), blood and the brain were collected and the radioactivities in them were counted by the gamma counter (Auto Well Gamma System ARC 300, Aloka) to determine the brain capillary volume. The true brain concentration (C_{brain}*) was calculated as follows:

$$C_{brain}^* = (C_{brain} - r \cdot RB \cdot Cp)/(1 - r)$$

where r (ml/g brain) and Cp are the brain capillary volume and the measured plasma concentration, respectively.

Assay of Ranitidine Concentration in Plasma, Blood and the Brain

For the determination of plasma concentration, 25 μl of plasma, 100 μl of 75 μM nizatidine as an internal standard, 100 μl of 5N 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 completely evaporated under reduced pressure at room temperature. The residue was dissolved with 100 μl of mobile phase and 50 μl were subjected to HPLC. For the determination of blood concentration, 200 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 30 μM nizatidine solution and 1 ml of saline were homogenized for 1 min on ice. One hundred μl of 0.5N NaOH were added to the homogenate and then extracted with 5 ml of CH_2Cl_2. Three ml of the organic phase were completely evaporated, dissolved with 100 μl of mobile phase, centrifuged at 10000 g. Fifty μl of supernatant was subjected to HPLC.

The HPLC apparatus was an LC-6A (Shimadzu, Kyoto, Japan) equipped with the SPD-6A spectrophotometer (Shimadzu). The column was a 4 × 250 mm stainless tube packed with the Senshu gel 7C_{18}H (Senshu, Tokyo, Japan). The mobile phase was of 5 mM NaH_2PO_4 and 5 mM tetramethylammonium chloride in 5% CH_3CN and pumped in at the rate of 2 ml/min. The column temperature was maintained at 30°C. Ranitidine was detected spectrophotometrically at 320 nm. The detection limits of ranitidine were 1 μM for plasma and blood, and 0.5 nmol/g for brain, respectively.

Data Analysis

Difference of the sample means were evaluated by one-way ANOVA followed by Student's *t* test or Welch's test. The incidence of convulsion after i.c. administration of ranitidine was compared by the Fisher's exact probability test.

RESULTS

Convulsive Threshold Concentration of Ranitidine in Mice with Experimental Acute Renal or Hepatic Dysfunction

Intraperitoneal pretreatment of mice with UN and CT induced the renal and hepatic dysfunction, respectively. The UN treated mice had a high plasma urea nitrogen and normal

Table I. Pathophysiological Changes of Experimental Renal or Hepatic Dysfunction in Mice

	Renal		Hepatic	
	Control (n = 5)	UN treated (n = 7)	Control (n = 5)	CT treated (n = 5)
GOT (IU/L)	170 ± 4.9	186 ± 36.9	184 ± 7.2	11840 ± 1489**
GPT (IU/L)	24 ± 6.3	17 ± 1.5	46 ± 11.6	8912 ± 1229**
Plasma urea nitrogen (mg/dl)	21 ± 0.4	396 ± 56.7***	24 ± 5.4	22 ± 3.6

Results are reported as mean ± S.E.

** Significantly different from corresponding control group ($P < 0.01$, Welch's test).

*** Significantly different from corresponding control group ($P < 0.001$, Welch's test).

Table II. Brain Capillary Volume and Blood to Plasma Partition Ratio of Ranitidine in Mice with Experimental Renal or Hepatic Dysfunction

	Renal		Hepatic	
	Control (n = 5)	UN treated (n = 5)	Control (n = 5)	CT treated (n = 5)
Brain capillary volume (ml/g)	0.014 ± 0.001	0.015 ± 0.001	0.014 ± 0.001	0.014 ± 0.001
Blood/plasma partition ratio	0.92 ± 0.04	0.84 ± 0.02	0.90 ± 0.05	0.86 ± 0.07

Results are reported as mean ± S.E.
No statistically significant difference can be seen between UN or CT treated mice and the corresponding controls.

GOT/GPT, while the CT treated mice had high GOT/GPT and normal plasma urea nitrogen (Table I). Blood content in the brain and the RB in UN or CT treated mice were not significantly different from those in corresponding control mice (Table 2). Table 3 shows the onset time, the total dose of ranitidine, the concentration of ranitidine in plasma and the brain and the brain/plasma concentration ratio at the initiation of convulsion. The onset time of convulsion of UN treated mice was significantly shorter ($P<0.001$, Student's t test), and the total dose was significantly lower ($P<0.001$) than those of control mice. On the other hand, ranitidine concentration in the brain and the brain/plasma concentration ratio at the onset of clonic convulsion were not significantly different, though the ranitidine concentration in plasma of UN treated mice was significantly higher than that of the control mice. In CT treated mice, no significant difference was observed in the onset time, the total dose, the concentration in plasma and the brain at the initiation of clonic convulsion.

Effect of Renal or Hepatic Dysfunction on Intrinsic Convulsive Potency of Ranitidine

Clonic and/or tonic convulsions were observed after i.c. administration of ranitidine. The incidence of clonic and tonic convulsion in UN or CT treated mice was not significantly different from corresponding control mice (Table 4).

DISCUSSION

Several reports describing H_2 antagonist-induced CNS disturbance, such as mental confusion, delirium and convulsion, suggested the individuals with renal and/or hepatic disease states may be at increased risk (5,6). Our retrospective analysis concluded that the decreased elimination capacity in renal disease and increased permeability through the blood-brain barrier and/or the blood cerebrospinal fluid barrier in hepatic disease state may be the mechanism for development of CNS toxicity from H_2 antagonists (6). Cimetidine- and ranitidine-induced convulsions have been reported after i.c. or i.p. administration of drug in mice (12–14), therefore mice were used to investigate the effect of renal or hepatic dysfunction on the neurotoxicity of ranitidine.

In the UN treated mice, the onset time of clonic convulsion was shorter than that of the control mice (Table 3). The higher plasma concentration in the UN treated mice should be due to the decreased renal elimination capacity of ranitidine. Since the brain concentration at the onset of clonic convulsion between the UN treated and the control mice was not significantly different, the toxic sensitivity to H_2 antagonists might be unaffected by renal dysfunction. In order to exclude the effect of pharmacokinetic alteration, we studied the effect of renal dysfunction on the incidence of convulsion after i.c. administration of ranitidine. We found that the intrinsic convulsive sensitivity was not affected by renal dysfunction (Table 4). In general, the reduction of the renal excretion capacity, the permeability of the blood-brain barrier and/or the blood-cerebrospinal fluid barrier permeability, accumulation of endogenous neurotoxic substances, etc., should be considered as risk factors for CNS during toxicity. Ramzan and Levy (15) reported that increased protein concentration in the CSF of ureter-ligated rats may be

Table III. Effect of Experimental Renal or Hepatic Dysfunction on Convulsive Threshold Concentration of Ranitidine in Mice

	Renal		Hepatic	
	Control (n = 5)	UN treated (n = 7)	Control (n = 5)	CT treated (n = 5)
Onset time of convulsion (min)	22.7 ± 1.7	9.3 ± 1.0***	21.4 ± 0.85	20.2 ± 1.3
Total dose (mg/kg)	299 ± 18	186 ± 17***	331 ± 7.6	295 ± 18.3
Plasma concentration (μM)	530 ± 47	752 ± 76*	579 ± 19	570 ± 37
Brain concentration (nmol/g)	2.11 ± 0.5	3.53 ± 0.76	4.39 ± 0.45	4.79 ± 0.69
Brain/Plasma ratio (%)	0.47 ± 0.08	0.55 ± 0.14	0.79 ± 0.08	0.84 ± 0.09

True brain concentration was calculated according to the equation in the text.
Results are reported as mean ± S.D.
* Significantly different from corresponding control group ($P < 0.05$, Student's t test).
*** Significantly different from corresponding control group ($P < 0.001$, Student's t test).

Table IV. Effect of Experimental Renal or Hepatic Dysfunction on Incidence of Convulsion Caused by i.c. Administration of Ranitidine in Mice

	Renal		Hepatic	
	Control (n = 20)	UN treated (n = 20)	Control (n = 20)	CT treated (n = 20)
Clonic and/or tonic convulsion (%)	65	60	60	50
Clonic convulsion (%)	40	35	35	40
Tonic convulsion (%)	45	40	35	45

due to the increased permeability of the blood-brain barrier and/or the blood-cerebrospinal fluid barrier. Increase in the plasma concentration of 1,3-dimethylurea, a dominant metabolite of theophylline, was shown to induce the neurotoxic convulsions in a patient with renal failure (16). The brain uptake of this compound is usually negligible in normal individuals. Accumulation of dialyzable neurotoxic components in the blood of uremic rats may additively increase the risk of neurotoxicity of some drugs. However, in this study, there was no significant difference between UN treated and control mice in terms of the brain uptake and intrinsic convulsive activity of ranitidine. This inconsistency these previous reports (15,16) may be due to the difference in the animal species and/or disease states tested. In the clinical situation, most patients with renal dysfunction suffer from anuria. Therefore, the uremic animal with ureteral ligation might be a more suitable model, but, unfortunately, 90% of mice die within 48 hr of bilateral ureteral ligation.

In the CT-treated mice, the onset time, total ranitidine dose, and the concentration in plasma and brain at the initiation of clonic convulsion were not different from those of control animals. Furthermore, there were no significant differences between the CT-treated mice and the control mice in the intrinsic convulsive incidence by i.c. administration. From these findings, acute hepatic dysfunction induced by CT treatment did not seem to bring about a pharmacokinetic/pharmacodynamic effect on ranitidine-induced convulsive neurotoxicity. Based on the retrospective analysis of pharmacokinetic and toxicodynamic data of H_2 antagonists in patients, we reported the possibility that renal and/or hepatic disease may be risk factors for neurotoxicity (6). In this study, however, we found no significant difference between CT treated and control mice in the brain uptake and the intrinsic convulsive activity of ranitidine. Some cases of H_2 antagonist-induced convulsion or mental confusion were reported in patients with hepatic cirrhosis or hepatoma, while no case reports could be found in patients with acute hepatitis (6). Therefore, further investigation may be required to evaluate the effect of chronic hepatic failure.

In this study, the threshold ranitidine concentration in brain was used to evaluate the effect of each disease state on manifestation on CNS side effects. In the previous report (6), we predicted the plasma concentration of ranitidine to induce mental confusion was in the range of 310–880 ng/ml in humans with normal renal/hepatic function, 200–600 times lower than the threshold plasma concentration in control mice (530–580 μM). Furthermore, the threshold CSF concentration of ranitidine in humans was assumed to be 53 ng/ml, 40–90 times higher than the threshold brain concentration in control mice. These discrepancies may be due to the nature of the response measured (mental confusion/clonic convulsion) and intrinsic species (human/mice) differences.

It is known that, in some drugs, the pharmacological effect on the CNS is not directly related to drug concentration in the brain and that the concentration in CSF may reflect the drug concentration at the site of action (18,19). Since infusion rate dependence of threshold brain concentration was not confirmed in this study, the possibility that the ranitidine concentration in the brain does not reflect the drug concentration at the site of action cannot be excluded.

Changes in protein binding in disease states are important factors in the tissue distribution of the drugs. The unbound fraction of several drugs in plasma increases in renal and hepatic failure (20,21). However, the change in the unbound fraction of H_2 antagonist should be negligible because the unbound fraction in normal mice is greater than 80% (22).

The risk of CNS toxicity of drugs with the low brain/plasma concentration ratios, such as H_2 antagonists, may be greatly enhanced by slight fluctuation in the permeability of the blood-brain barrier and/or the blood-cerebrospinal fluid barrier in disease states. Furthermore, the toxicity of drugs may be enhanced by endogenous toxic substances generated by disease states, resulting in the appearance of unexpected side effects. Therefore, in order to predict the occurrence of CNS side effects of newly developed H_2 blockers, it is essential that one investigate change in the permeability of the blood-brain barrier and/or the blood-cerebrospinal fluid barrier and the neurotoxic sensitivity in animals with appropriate disease state models.

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