Defect of Urinary Concentration Capacity in Cephaloridine-Administered Rats

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The nephrotoxic effects of cephaloridine on the renal concentration capacity were examined in rats that had received a single intravenous injection of cephaloridine (100 or 500 mg/kg body weight). An increase in urinary volume and a decrease in urinary osmotic pressure were observed continuously from the first to the sixth day in the 500 mg/kg group, but not in the 100 mg/kg group. In addition, rises in plasma levels of arginine-vasopressin were observed in the 100 and 500 mg/kg groups. Furthermore, responsiveness to exogenous arginine-vasopressin was investigated in the cephaloridine-administered rats under ethanol anesthesia. The injection of arginine-vasopressin caused relatively slight responses, in terms of the urine flow and urinary osmolality, in the cephaloridine-administered group in comparison to the control group. These findings indicate that cephaloridine induces a vasopressin-resistant polyuria, and strongly suggest that cephaloridine does damage the collecting tubules in the kidney.

Keywords——cephaloridine; glucosuria; polyuria; hypotonic urine; water transport system; vasopressin; nephrogenic diabetes insipidus; kidney; toxicity; rat

Introduction

Cephaloridine (CER), a broad-spectrum cephalosporin antibiotic, is known to produce nephrotoxicity that is characterized by glucosuria, proteinuria, and other features,[1] and its main toxic effect has been histologically proved to be on the proximal tubules in addition to the descending limbs of Henle's loop, but not on the distally located tubules including the ascending limbs of Henle's loop, the distal tubules and the collecting tubules.[1b,2]

In the present experiments to investigate toxic effects of CER, it was found that CER caused glucosuria without hyperglycemia and polyuria with hypotonic urine. This glucosuria without hyperglycemia was considered to be attributable to the injury in the proximal tubules. However, as regards the polyuria with hypotonic urine, the following two possibilities were considered as etiological causes: an osmotic diuresis induced by glucose which was not reabsorbed in the proximal tubules, and a defect of ability to reabsorb water along the nephrons.

Concerning the polyuria with hypotonic urine, we considered that CER might injure the collecting tubules as well as the proximal tubles. Based on this hypothesis, we endevored to investigate the relationship between the water transport and the responsiveness to vasopressin in the kidneys of CER-administered rats. In addition, the proximal tubular injury induced by CER was investigated, focussing on the proximal tubular reabsorption of glucose.

Experimental

Chemicals——CER (Keflodine) was purchased from Shionogi Co., Ltd.(Osaka, Japan), and arginine-vasopressin (AVP) from the Protein Research Foundation (Osaka, Japan). For AVP assay, "Immuno Nuclear

Arginine Vasopressin by RIA Kit" was obtained from Immuno Nuclear Co. (MINN, U.S.A.). All other reagents used in this study were commercial products of the highest grade available.

General Procedure——Male Wistar rats weighing 200—220 g (Sankyo Labo Service Co.; Tokyo, Japan) were housed in a temperature (25 ± 2 °C), humidity (relative humidity, $55 \pm 10\%$), and light (12 h light, 12 h dark) controlled room, and allowed free access to water and standard diet pellet (MF; Oriental Yeast Co.; Chiba, Japan). In order to avoid diurnal changes, dosing of the rats and other operations were done between 10 to 11 a.m. The rats received a single intravenous injection of CER in a suitable volume of saline (2 ml/kg body weight) through the tail vein, with the doses being 100 and 500 mg/kg body weight. The control rats received the same volume of saline. The period from 0 to 24 h following the CER-administration was designated as the first day.

Experimental Design——Protocol 1: Changes of the following items were investigated: body weight gain, food and water consumptions, urinary excretion of glucose, urinary excretion of water (urinary volume), and urinary osmotic pressure. The control and the CER-administered rats were placed individually in metabolism cages (KN-646, B-1 type; Natsume Co., Tokyo, Japan) designed for separation of urine and feces, and were given free access to water and diet. The urine was collected every 12 h in a flask in which 5 ml of water-saturated mineral oil and 50 μl of formalin had been put beforehand. The urine obtained was centrifuged (1700 × g, 10 min, 4 °C), and the supernatant was used for determinations of glucose and osmotic pressure.

Protocol 2: Concentrations of plasma AVP and blood glucose, and serous osmotic pressure were determined in the control and the CER-administered rats, at 48 and 96 h after the CER-administration. The rats were killed with a guillotine. Blood issuing from the trunk was collected. A 0.1 ml aliquot of blood was immediately mixed with 1 ml of 0.33 M perchloric acid as used for determination of glucose.[3a] Another aliquot (approximately 0.2 ml) was used for determination of serous osmotic pressure. The residual blood was immediately treated with ethylenediaminetetraacetic acid tetrasodium salt (final concentration, approximately 7.5 mg/5 ml), and centrifuged at 760 × g for 15 min at 4 °C. The separated plasma was used for AVP assay.

Protocol 3: To examine renal water handling, responses to AVP were investigated in the control and the 500 mg/kg groups, 96 h after the CER-administration. The procedure was based on the method of Koyama et al.[3b] The rats were anesthetized by oral administration of ethanol to inhibit secretion of endogenous vasopressin. A dose of 10 ml/kg body weight of 24% ethanol was given orally 3 times every 30 min through a gastric tube. The ethanol-anesthetized rat was intubated for free respiration, then the left femoral vein was catheterized with polyethylene tubing (PE-50). Throughout each experiment, a hypotonic solution consisting of 1.2% ethanol, 1.7% glucose and 0.3% NaCl, was injected through the femoral catheter at a rate of 10 ml/kg body weight as a prime, followed by a constant infusion of the hypotonic solution at a rate of 0.5 ml/kg body weight/min. After a small midsection in the lower abdominal wall, the urinary bladder was exposed and polyethylene tubing (PE-190) was inserted into the bladder through the incised wall in order to make a bladder fistule. An equilibration period of 3 h, was allowed to obtain a constant urine flow. At this time point, collection of the urine was started. After collection of the urine for 10 min, AVP was injected through the femoral catheter, with the doses being 199.5 and 1995 pg/kg body weight. After the AVP injection, the urine was collected at intervals of 10 min. The excreted water volume (urine flow) and osmotic pressure were determined.

Analytical Procedures——Urinary volume was measured by weighing the urine on the basis of 1 ml = 1 g. Osmotic pressure in urine and serum was measured by using an Automatic Micro-Osmometer (Hermann Roebling Co., Berlin, F.R.G.) in a volume of 50 μl. Glucose in urine and blood was assayed by the method of Bergmeyer et al.,[3a] using a HITACHI-320 spectrophotometer (Hitachi Co., Tokyo, Japan). Plasma AVP concentration was determined by using an "Immuno Nuclear Arginine-Vasopressin by RIA Kit," following extraction of AVP from the plasma by the use of octadecasilyl-silica. Gamma counting was done by using a Auto Well Gamma System ARC-305 (Aloka Co., Ltd.; Tokyo, Japan).

Statistics——Results were represented as the mean $\pm$ S.E. Statistical significance was assessed by the use of Student's t-test for paired samples, p values of less than 0.05 being considered significant.

Results

Experiments of Protocol 1

In the first series of experiments, changes of body weight gain, food and water consumptions, urinary excretion of glucose, urinary volume and urinary osmotic pressure were investigated for 10 d.

The control and 100 mg/kg groups showed similar body weight gains, while the 500 mg/kg group showed a suppression of body weight gain in the first, fourth, fifth and sixth days in comparison with the control group.

In terms of food consumption, the 100 and 500 mg/kg groups showed a decrease only in
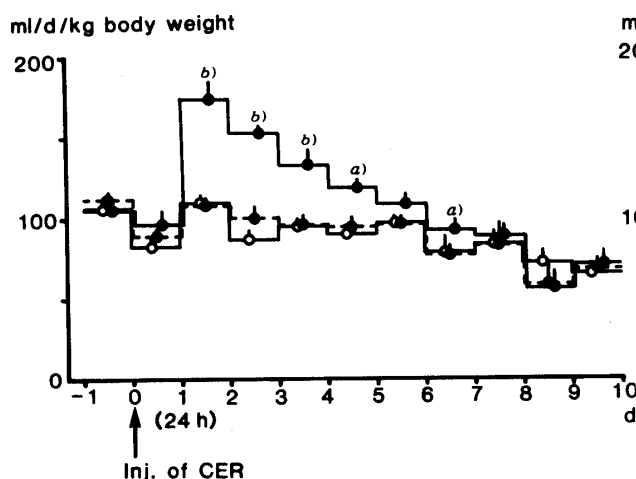
ml/d/kg body weight

Fig. 1. Changes in Water Consumption Induced by Administration of CER

Points and vertical bars represented the mean ± S.E. The number of rats in each group was 6. The values in the 100 and 500 mg/kg groups at each time were statistically compared with those of the control group: a) $p<0.05$, b) $p<0.01$. Abbreviations: control, control group; CER, cephaloridine; CER 100 mg/kg, group administered 100 mg CER per kg body weight; CER 500 mg/kg, group administered 500 mg CER per kg body weight; inj. of CER, injection of CER. ●---●, control; ○—○, CER 100 mg/kg; ●—● CER 500 mg/kg.
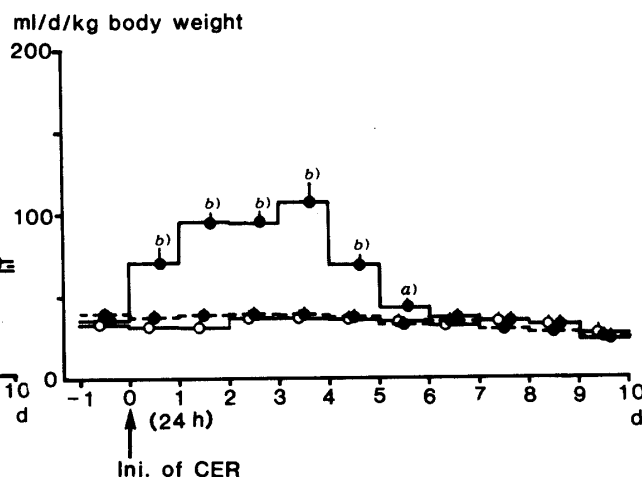
ml/d/kg body weight

Fig. 2. Changes of Urinary Volume Induced by Administration of CER

Details are as given in the legend to Fig. 1. ●---●, control; ○—○, CER 100 mg/kg; ●—●, CER 500 mg/kg.

the first day as compared with the control group.

Figure 1 shows the results on water consumption. The water consumption in the 500 mg/kg group showed no significant difference from that of the control group on the first day, while the water consumption was maximally increased on the second day. Thereafter, it gradually reverted toward the control level, though showing significant increases as compared to the control group up to the seventh day. The 100 mg/kg group showed no significant change in comparison with the control group throughout the period.

Figure 2 shows the results on urinary volume. The urinary volume in the 500 mg/kg group gradually increased from the first day, and reached its maximal level on the fourth day. Thereafter, it gradually decreased, and reverted to the control level on the seventh day. The 100 mg/kg group showed no significant change, as compared with the control group, throughout the period.

Figure 3 shows the results on the urinary osmotic pressure. The urinary osmotic pressure in the 500 mg/kg group fell to less than 1500 mOsm/kg from the first day, with this level being maintained up to the fourth day: the maximal fall was observed on the fourth day. Thereafter, the urinary osmotic pressure gradually rose, and was restored to the control level on the seventh day. The 100 mg/kg group showed no significant change as compared with the control group throughout the period.

Figure 4 shows the results on urinary glucose excretion. The urinary glucose excretion in the 500 mg/kg group increased from the first day, and reached its maximal level on the second day. Thereafter, it gradually decreased, and had almost reverted to the control level on the sixth day. The 100 mg/kg group showed no significant change in comparison with the control group throughout the period.

Experiments of Protocol 2

In the second series of experiments, using other control and CER-administered rats,
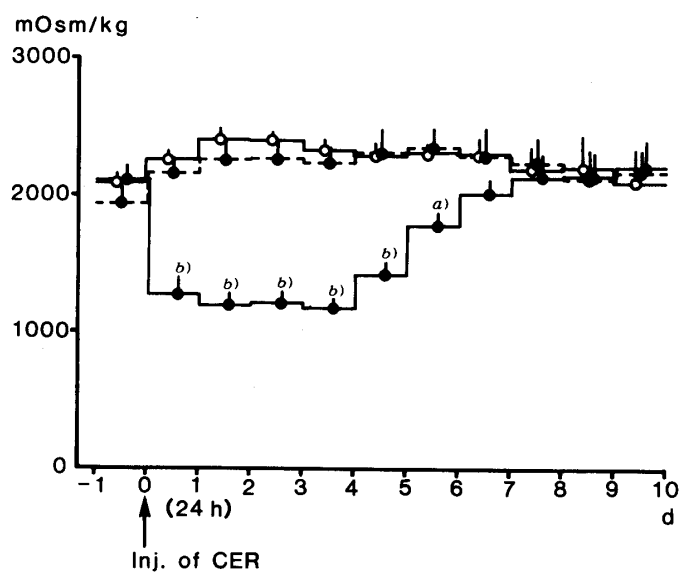
mOsm/kg

Fig. 3. Changes of Urinary Osmotic Pressure
Induced by Administration of CER

Details are as given in the legend to Fig. 1.
●---●, control; O—O, CER 100 mg/kg; ●—●,
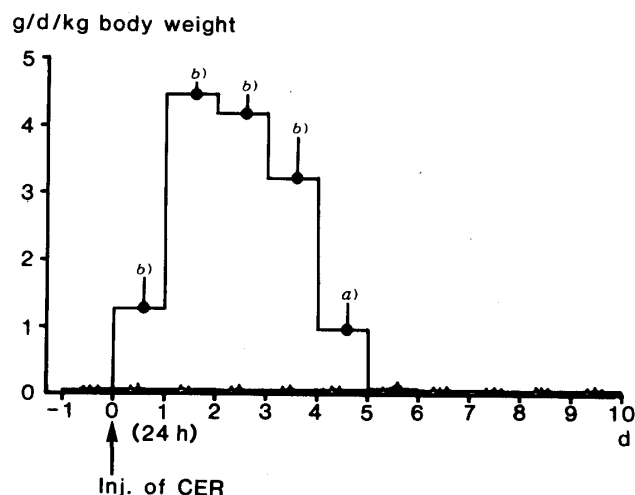CER 500 mg/kg.

g/d/kg body weight

Fig. 4. Changes of Urinary Glucose Excretion
Induced by Administration of CER

Details are as given in the legend to Fig. 1.
●---●, control; O—O, CER 100 mg/kg; ●—●,
CER 500 mg/kg.

TABLE I. Concentrations of Plasma AVP and Blood Glucose, and Serous
Osmotic Pressure in Control and CER-Administered Rats

	Group	48 h	96 h
Plasma AVP	Control	1.7 ± 0.1	1.6 ± 0.2
(pg × ml)	CER 100 mg/kg	$20.4 \pm 3.9^{a)}$	$14.7 \pm 1.4^{a)}$
	CER 500 mg/kg	$17.3 \pm 3.1^{a)}$	$28.2 \pm 2.7^{a)}$
Blood glucose	Control	97.3 ± 1.8	102.3 ± 0.9
(mg/dl)	CER 100 mg/kg	95.7 ± 2.7	101.0 ± 2.3
	CER 500 mg/kg	99.1 ± 1.4	99.9 ± 3.0
Serous osmotic pressure	Control	292.6 ± 0.9	291.8 ± 1.2
(mOsm/kg)	CER 100 mg/kg	295.0 ± 2.4	293.2 ± 1.3
	CER 500 mg/kg	296.4 ± 1.4	293.8 ± 1.3

Rats were used at 48 and 96 h after CER-administration. The values at each time point were statistically
compared with the values of the control group: a) $p < 0.01$. The number of rats in each group was 5. Other
details are as in the legend to Fig. 1.

concentrations of plasma AVP and blood glucose, and serous osmotic pressure, were
determined at 48 and 96 h, since maximal changes in urinary glucose excretion, urinary
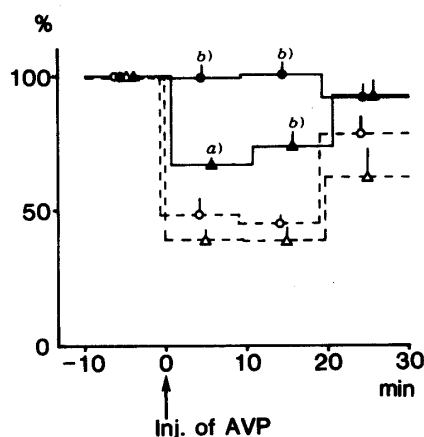volume and urinary osmotic pressure were observed, as represented in the experiments of

Fig. 5. Time-Dependent Changes of Urine Flow Caused by AVP Injection in Control and CER-Administered Groups

O---O, AVP injection at 199.5 pg/kg in control group; △---△, AVP injection at 1995 pg/kg in control group; ●—●, AVP injection at 199.5 pg/kg in CER 500 mg/kg group; ▲—▲, AVP injection at 1995 pg/kg in CER 500 mg/kg group.

Abbreviations: AVP, arginine-vasopressin; inj. of AVP, injection of AVP. Rats were used at 96 h after CER-administration. AVP at a dose of 199.5 or 1995 pg/kg was injected into the control and the CER-administered rats. Rates (%) of urine flow in every 10 min after AVP injection are represented based on values before the AVP injection. The values at each time point were statistically compared with those (O---O) of the control rats which were administered AVP at the lower dose of 199.5 pg/kg: a) $p < 0.05$; b) $p < 0.01$. The number of rats in each group was 5. Other details are as given in the legend to Fig. 1.
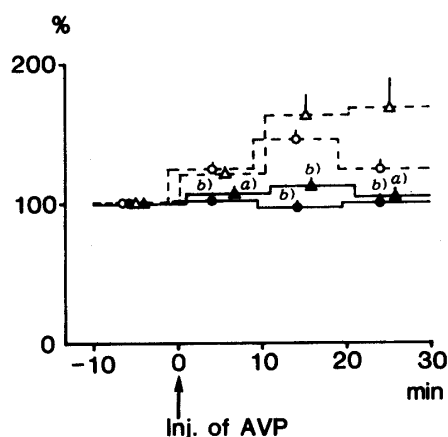

Fig. 6. Time-Dependent Changes of Urinary Osmotic Pressure Caused by AVP Injection in Control and in CER-Administered Groups

Details are as given in the legend to Fig. 5.

protocol 1, in the second or fourth day after the CER-administration. Table I shows the results. As regards the concentration of blood glucose and serous osmotic pressure, no significant differences were observed among the control and CER-administered groups at 48 and 96 h. In contrast, plasma AVP in the 100 and 500 mg/kg groups was more than 10 times that of the control group, at 48 and 96 h. The highest value was observed at 96 h in the 500 mg/kg group, but at 48 h in the 100 mg/kg group.

Experiments of Protocol 3

As described above, the maximal rise (28.2 ± 2.7 pg/ml) in plasma AVP concentration was observed at 96 h in the 500 mg/kg group. An exogenous AVP dose of 1995 pg/kg was expected to correspond to the above maximal plasma AVP concentration, assuming that the plasma volume is 7% of body weight.[3b] In addition, another dose of 199.5 pg/kg (1/10 of 1995 pg/kg) was employed as a lower dose. In the third series of experiments, the responsiveness to exogenous AVP, focussing on the urinary concentration capacity, was investigated by loading ethanol-anesthetized rats of the 500 mg/kg group with hypotonic saline at 96 h after the CER-administration.

Figures 5 and 6 show the results on urinary volume and urinary osmotic pressure, respectively, based on the values before the AVP injection. The values (mean $\pm$ S.E., $n = 12$) before the AVP injection were as follows: urinary volume (ml/min/kg body weight), 0.426 ± 0.041 in the CER non-administered groups and 0.495 ± 0.034 in the CER 500 mg/kg groups; urinary osmotic pressure (mOsm/kg), 179.8 ± 4.6 in the CER non-administered groups and 183.5 ± 3.8 in the CER 500 mg/kg groups. In the CER non-administered groups, the AVP injections dose-dependently caused falls in the urinary volume and rises in the

urinary osmotic pressure: these changes were more marked at the higher AVP dose than at the lower AVP dose. In the 500 mg/kg groups, the AVP injection at the lower dose caused hardly any change in urinary volume or urinary osmotic pressure. The AVP injection at the higher dose caused falls in the urinary volume and rises in the urinary osmotic pressure, though these changes were smaller than in the CER non-administered groups at both AVP doses.

Discussion

It is known that the glucose filtered through the glomeruli is reabsorbed in the proximal tubules.[4] The fluid filtered through the glomeruli is reabsorbed along regions ranging from the proximal convoluted tubules to the descending limbs of Henle's loop,[5] and the final volume and osmolality of the excreted urine ultimately depend on the state of the collecting tubules, in which vasopressin causes an increase in water permeability of the luminal membrane of the cells.[6] In this study, the effects of CER on renal tubular reabsorption of glucose and renal concentration ability of urine were mainly investigated as indices for evaluation of its toxicity to the proximal tubules and the collecting tubules, respectively.

The experiments in protocols 1 and 2 showed that the plasma glucose concentrations in the CER-administered groups remained within the control levels throughout the whole process, even on the second day, when the urinary excretion of glucose reached the maximal level in the 500 mg/kg group. This finding of glucosuria without hyperglycemia indicates that the CER-administration resulted in a decrease of the proximal tubular reabsorption capacity for glucose. This is quite natural, because the proximal tubular injuries induced by CER are considered to cause such a physiological dysfunction in the proximal tubules.[1a,b] We consider that the glucosuria without hyperglycemia is a feature of the CER-induced nephrotoxicity, and that the investigation of urinary glucose excretion could be a useful tool for evaluation of proximal tubular injury induced by nephrotoxic compounds such as CER, provided that the plasma glucose level remains normal.

In addition, an increase in urinary water excretion (polyuria) and a decrease in urinary osmotic pressure (hypotonic urine) were observed in the 500 mg/kg group: the highest urinary water excretion was observed on the fourth day, while urinary osmotic pressure showed a rough plateau from the first to the fifth day, with the lowest value on the fourth day. In contrast with the finding that the glucosuria was observed with a peak in the second day, the polyuria with hypotonic urine peaked on the fourth day, when the glucosuria had more or less recovered. In the presence of proximal tubular injuries, the polyuria could be caused by a decrease in the water reabsorption capacity, or by osmotic diuresis due to the unreabsorbed glucose in the proximal tubules. However, this dissociation in the time courses of the glucosuria and the polyuria with hypotonic urine suggested that CER impaired not only the proximal tubules, but also other portions of tubules, that is, the distally located tubules. As regards the severity and period of the injuries, the single intravenous administration of CER at a dose of 500 mg/kg seemed to have relatively prolonged toxic effects on the distally located tubules in comparison to the proximal tubules. Further dose-related studies would seem to be worthwhile.

In order to investigate further the possibility that dysfunction of concentration ability can be ascribed to distally located tubular impairment, the experiments in protocols 2 and 3 were carried out focussing on a system regulated by vasopressin in the collecting tubules. Plasma AVP level and serous osmolality were determined at 48 and 96 h, when peaks of glucosuria and polyuria with hypotonic urine were respectively observed in the 500 mg/kg group. No change in serous osmolality was observed in the 100 and 500 mg/kg groups. In contrast, rises in the plasma AVP level were found in the two groups: its levels were higher at 48 h than at 96 h in the 100 mg/kg group, and, conversely, higher at 96 h than at 48 h in the

500 mg/kg group. In the 100 mg/kg group, no significant change of investigated items other than the plasma AVP level was found. This fact suggests that the single intravenous injection of CER, at the dose of 100 mg/kg, causes slight (no readily detectable) damage to the collecting tubules, and that the rises of concentrations of plasma AVP compensate for the dysfunction in the impaired collecting tubules. Also, the results at the higher dose of 500 mg/kg suggest that CER does cause a relatively severe damage to the collecting tubules: though the plasma AVP level rose to more than 10 times that of the control group, the plasma AVP hardly seemed to control the water reabsorption in the collecting tubules.

For more direct demonstration of this dysfunction in the AVP-dependent regulation system of water reabsorption, responsiveness to exogenous AVP was investigated. It is convenient that the secretion of endogenous AVP is suppressed by treatments such as administration of ethanol, phenytoin, etc.[7] For this purpose, as well as anesthesia,[3b] ethanol was administered to the rats at 96 h after CER-administration at the dose of 500 mg/kg: at this time point, the increase of plasma AVP level and polyuria with hypotonic urine were most marked. The two AVP doses (199.5 and 1995 pg/kg) were employed based on the plasma level of 28.2 pg/ml at 96 h in the CER 500 mg/kg group: the plasma level of 28.2 pg/ml corresponds to a single intravenous AVP injection of 1995 pg/kg, assuming that the plasma volume is 7% of the body weight. Accordingly, the lower dose of 199.5 pg/kg could be considered to correspond to a plasma level of 2.82 pg/ml (1.76 times the control level). This lower dose of 199.5 pg/kg caused a fall of urinary volume to nearly 50% and an elevation of urinary osmotic pressure to more than 140% in the control group. In comparison with the control, the 500 mg/kg group showed hardly any response at the lower AVP dose, and a moderate response at the higher AVP dose. This finding indicates that the renal responsiveness in terms of water reabsorption in the collecting tubules in the 500 mg/kg group was almost lost at 1.76 times the plasma AVP level in the control rats. This state of increase in water excretion induced by loss of responsiveness to AVP resembles the disease "nephrogenic diabetes insipidus," which is characterized by polyuria and loss of ability of the kidney to concentrate urine when fluid intake is restricted.[8a,b] In this disease, the inability of the kidney to concentrate urine has been considered to be ascribable to unresponsiveness of the epithelial cells of the collecting tubules to either endogenous or exogenous vasopressin.[8c] Thus, CER may cause a drug-induced "nephrogenic diabetes insipidus." In addition, the increase in water consumption in the CER-administered rats in this study may be regarded as a response to the loss of body fluid for maintenance of homeostasis.

Lastly, we cannot completely exclude the possibility that the defect of urinary concentration capacity caused by CER-administration was secondary to the proximal tubular damage, because histological precedents[1b,2] in the literature on acute toxicity in rats indicate that CER injured regions ranging the proximal convoluted tubules to the descending limbs of Henle's loop, not the distally located tubules ranging from the ascending limbs of Henle's loop to the collecting tubules: there were alterations of the proximal tubular brush-border at 200 mg/kg i.v.,[1b] severe nuclear pyknosis, cytoplasmic disintegration and patchy loss of the brush-border in the proximal tubules at 500 mg/kg i.v.,[2b] and widespread areas of necrosis and hydropic changes in the proximal tubules and the descending limbs of Henle's loop at 1000 mg/kg i.v.[2b] Although these studies documented the injuries in the proximal tubules and the descending limbs of Henle's loop, there has been no histological report that CER damaged the collecting tubules. Therefore, we consider that further investigations at the nephron level in the rat kidney, (for example, measurement of cyclic adenosine monophosphate in nephron segments), are needed for elucidating the mechanism(s) of the above-mentioned loss of responsiveness to vasopressin in CER-induced nephrotoxicity.

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