Histopathological Study of Kidney Abnormalities in an Experimental SIADH Rat Model and Its Application to the Evaluation of the Pharmacologic Profile of VP-343, a Selective Vasopressin V<sub>2</sub> Receptor Antagonist

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The aim of this work was to investigate histopathologically the relationship between the syndrome of inappropriate secretion of antidiuretic hormone (SIADH) and kidney abnormalities and the therapeutic efficacy of VP-343 ((N-[4-[(2S,3aR)-2-hydroxy-2,3,3a,4-tetrahydropyrrolo[1,2-a]quinoline-5(1H)-yl]phenyl]-4'-methyl[1,1'-biphenyl]-2-carboxamide], a selective vasopressin V<sub>2</sub> receptor antagonist, in an experimental SIADH rat model. In the model, which was prepared by continuously administering 1-desamino-8-arginine vasopressin (DDAVP), histopathologic abnormalities, such as dilatation of tubules, basophilic changes in tubules, inflammatory cell infiltration, and mineralization were found in the kidney, accompanied by significant increases in the relative weight of the kidney, lung, liver, adrenal gland, and heart. VP-343 was shown to be effective in protecting the kidney from the histopathologic abnormalities and to normalize the relative weight of the kidney and several common pathophysiologic features, such as hyponatremia, hyposmolality of plasma, hyperosmolality of urea, and oliguria, as described previously.

These results demonstrate the occurrence of histopathologic abnormalities in the kidney and the efficacy of VP-343 in improving abnormalities in the DDAVP-induced SIADH rat model.

Key words SIADH; VP-343; histopathology; vasopressin; kidney

The syndrome of inappropriate secretion of antidiuretic hormone (SIADH) is suggested to be caused by disorders of the central nervous system, a variety of malignant tumors, intrathoracic disorders, and a number of drugs.1–5 One of the criteria for SIADH has been “normal kidney function,”5 and there have been very few reports on the relationship between SIADH and abnormalities in the kidney. Common features of SIADH are hyponatremia, serum hyposmolality of plasma, hyperosmolality of urea, and oliguria, suggesting the undesirable influences on the circulatory system. Overloading of the kidney, which particularly influences the circulatory system, could cause abnormalities of kidney function. Therefore it is of interest to study the relationship between SIADH and kidney abnormalities.

The possible involvement of vasopressin in albumin urea level and hypertrophy of the kidney in diabetic nephropathy, and an increased vasopressin release in glomerular sclerosis have been reported.6,7 Vasopressin antagonists were reported to affect the levels of triglyceride, systolic blood pressure, serum creatinine, and the histopathologic index of glomerular sclerosis in progressive glomerulosclerosis in spontaneously hypercholesteremic rats.8,9 Therefore vasopressin antagonists could be expected to exhibit a therapeutic effect on the kidney in SIADH rats.

We previously reported the effects of VP-343, a novel selective vasopressin V<sub>2</sub> receptor antagonist, on common features in the SIADH rat model, e.g., changes in urine volume, serum levels of sodium, calcium, chloride, and cholesterol, serum and urine osmolality, etc.10 This paper dealt with the relationship between SIADH and kidney abnormalities and demonstrated the therapeutic efficacy of VP-343, based on the kidney histopathologic studies, in the 1-desamino-8-arginine vasopressin (DDAVP)-induced SIADH rat model.

MATERIALS AND METHODS

Materials DDAVP was obtained from Funakoshi Co., Ltd. VP-343 was synthesized as described previously.11 Commercial, nutritionally balanced liquid diet was obtained from Japan Clea Co., Tokyo, Japan. Arabic gum (AG) was obtained from Wako Pure Chemical Industry, Ltd.

Animals Male Sprague-Dawley rats (Charles River Japan, Inc.) were used. Animals were housed in communal cages and maintained on a 12-h light/dark cycle with food and water available ad libitum.

Animal Models and Experimental Protocol Animals were given liquid diet throughout the experimental period. The experimental procedures were based on the method described previously.10 The SIADH group was divided into SIADH/control and SIADH/VP-343 groups. DDAVP was administered by an osmotic minipump implanted subcutaneously; in the normal group saline was infused similarly. On day 6 postimplantation, the normal group and SIADH/control group received 5% AG orally, and the SIADH/VP-343 groups received VP-343 0.1, 0.3, 1, and 3 mg/kg orally, respectively. The rats were administered 5% AG or VP-343 orally once a day in a volume of 2 ml/kg from day 6 to 14.

On day 15, the heart, liver, lung, right kidney, and right adrenal gland were removed, and then each organ was weighed. Kidneys were fixed with 10% neutrally buffered formalin solution, and histopathologic examination was performed. Blood samples were collected while removing the organs. Serum samples were measured for sodium levels using a flame photometer.
On day 15, 24-h collected urine was measured for urine volume, and then urine osmolarity was measured using the freezing point depression method with an osmometer (Auto & STAT OM-6030, Kyoto Daiichi Kagaku, Japan).

**Histopathologic Examination** Kidneys fixed in 10% neutrally buffered formalin solution were embedded in paraffin and sectioned at a thickness of about 2 μm. The sections were stained with hematoxylin and eosin for light microscopic observation. Abnormal findings in kidneys were scored based on the following scales.

1. **Dilatation of Tubules:** −, no abnormal changes (normal); ±, slight (dilatation of tubule mainly in the medulla); +, mild (dilatation of tubule in the medulla and a limited area of the cortex); ++, moderate (dilatation of tubule in the medulla and cortex).
2. **Basophilic Changes in Tubules:** −, no abnormal changes (normal); ±, slight (minimal basophilic changes in tubule in 2—3% of sections); +, mild (basophilic changes in tubule in a narrow area in 3—10% of sections); ++, moderate (basophilic changes in tubule in a wide area in 20—50% of sections); ++++, severe (basophilic changes in tubule in a wider area in 50—70% of sections).
3. **Mineralization:** Mineralization was found in medullary tubules. −, no abnormal changes (normal); ±, slight (1—9 areas of mineralization); +, mild (10—20 areas of mineralization); ++, moderate (more than 21 areas of mineralization).
4. **Inflammatory Cell Infiltration:** −, no abnormal changes (normal); ±, slight (inflammatory cells slightly infiltrating tubule section); +, mild (inflammatory cells clearly infiltrating tubule in a limited area of the section).

**Statistical Analysis** All experimental results were expressed as the mean±S.E.M. Statistical analysis between the normal group and control group was performed using Student’s t test or Aspin–Welch’s test. Statistical analysis between the SIADH/control group and SIADH/VP-343 groups was performed using the Williams or Williams–Shirley test. The level of significance was taken as p<0.05.

**RESULTS**

**Histopathologic Examination** The results are shown in Table 1 and photomicrographs 1—12 in Fig. 1. No abnormal histopathologic findings in the kidneys were observed in the normal group (photomicrographs 1 and 7). In the SIADH/control group, histopathologic findings, such as dilatation of tubules, basophilic changes in tubules, inflammatory cell infiltration, and mineralization were observed (photomicrographs 2 and 8). The scores ranged from − to +++. In the SIADH/VP-343 group, no inflammatory cell infiltration was seen at VP-343 doses of 0.1, 0.3, 1, and 3 mg/kg (photomicrographs 3—6 and 9—12). The scores for dilatation of tubules, basophilic changes in tubules, and mineralization ranged from − to ++ at VP-343 doses of 0.1 and 0.3 mg/kg (photomicrographs 3, 4, 9, and 10), and from − to + at VP-343 doses of 1 and 3 mg/kg (photomicrographs 5, 6, 11, and 12).

**Organ Mass** The relative weight of the kidney, lung, liver, adrenal gland, and heart in the SIADH/control group was significantly greater than that in the normal group. VP-343 0.1—3 mg/kg significantly and dose-dependently normalized the relative weight of the kidney. Slight normalization was observed in the weights of the adrenal gland and heart, although statistically, the change was not significant. No normalization was observed in lung and liver weights (Fig. 2).

**Serum Sodium and Serum Osmolarity** The serum sodium level in the normal group on day 15 was 141.4±0.3 mEq/l. In contrast, the serum sodium levels in the SIADH/control group on day 15 were significantly lower than those in the normal group. VP-343 0.1—3 mg/kg p.o. elevated the serum sodium levels dose-dependently on day 15 (Fig. 3A).

The serum osmolarity level in the normal group on day 15 was 313±1.6 mOsm/kg; in contrast, that in the SIADH/control group was 253±3.1 mOsm/kg, which was significantly lower than that in the normal group. VP-343 restored the serum osmolarity levels dose-dependently on day 15 (Fig. 3B).

**Urine Volume and Urine Osmolarity** In the normal group, 24-h urine volume on day 15 was 63.9±5.1 ml/kg; in contrast, that in the SIADH/control group was 39.4±4.4 ml/kg, which was significantly lower than that in the normal group. VP-343 increased urine volumes significantly and dose-dependently on day 15, as shown in Fig. 4A.

The urine osmolarity level in the normal group on day 15 was 362.6±21 mOsm/kg; in contrast, the SIADH/control group had a significantly higher osmolarity of 924.8±108.8 mOsm/kg on day 15. In the VP-343 groups, VP-343 decreased urine osmolarity, significantly in a dose-dependent manner (Fig. 4B).

**DISCUSSION**

The critical findings for the diagnosis of SIADH are as follows; 1) hyponatremia; 2) hyposmolarity of plasma; 3)
Fig. 1. Photomicrographs of Histopathology of Tubules in Rat Kidney Medulla (1—6) and Cortex (7—12)

1. normal; 2. SIADH/control; 3. SIADH/VP-343, 0.1 mg/kg; 4. SIADH/VP-343, 0.3 mg/kg; 5. SIADH/VP-343, 1.0 mg/kg; 6. SIADH/VP-343, 3.0 mg/kg; 7. Normal; 8. SIADH/control; 9. SIADH/VP-343, 0.1 mg/kg; 10. SIADH/VP-343, 0.3 mg/kg; 11. SIADH/VP-343, 1.0 mg/kg; 12. SIADH/VP-343, 3.0 mg/kg.
Fig. 2. Effect of VP-343 on Relative Organ Weight of the Kidney, Adrenal Gland, Heart, Liver, and Lung in the Experimental SIADH Rats
Values are means±S.E.M. from 6—8 animals. Statistical comparisons between the normal and SIADH/control groups were made using two-way analysis of variance followed by the Aspin–Welch's test or Student's t-test (**p<0.01, *p<0.05). Statistical comparison between the SIADH/control and SIADH/VP-343 groups were made using two-way analysis of variance followed by the parametric or nonparametric Dunnett's multiple comparison test (##p<0.01, #p<0.05).

Fig. 3. Effect of VP-343 on Serum Sodium Concentration (A) and Serum Osmolarity (B) in the Experimental SIADH Rats
Blood samples were collected from the abdominal aorta on the day after final administration of VP-343 (day 15). Values are means±S.E.M. from 6—8 animals. Methods for statistical analyses and symbols for p values are the same as described in Fig. 2.

Fig. 4. Effect of VP-343 on Urine Volume (A) and Urine Osmolarity (B) in the Experimental SIADH Rats
Twenty-four hours accumulated urine was collected on the day after final administration of VP-343 (day 15). Values are means±S.E.M. from 6—8 animals. Methods for statistical analyses and symbols for p values are the same as described in Fig. 2.
continued renal excretion of sodium although taking no diuretics; 4) absence of clinical evidence of fluid volume depletion; 5) urine osmolarity greater than appropriate considering the plasma osmolarity; 6) normal renal function; 7) normal adrenal function; and 8) normal thyroid function without findings of exhaustion, anorexia, and dehydration. 

However, reports and studies dealing with SIADH have not strictly shown all of these findings; the criteria adopted as the minimum findings defining SIADH have included hyponatremia, serum hyposmolarity, and urinary hyperosmolarity characterized by continued sodium excretion. As described above, normal renal function is one feature of SIADH, and there have been very few studies on the relationship between SIADH and abnormalities of the kidney. We previously reported the therapeutic efficacy of VP-343 in SIADH with the SIADH rat model which fulfilled some of the features of SIADH including hyponatremia, serum hyposmolarity, oliguria, and hyperosmolarity of urine. The oliguria and hyperosmolarity of urine should overload kidney function and cause histopathologic abnormalities in the kidney due to the continuous reabsorption of water.

The above prompted us to study the relationship between SIADH and abnormalities of the kidney in the SIADH rat model, and thus we performed histopathologic examinations of the kidney. As shown in Table 1 and photomicrographs 2 and 8 in Fig. 1, in the SIADH/control group, no abnormal histopathologic findings were noted with regard to the corporcles and blood vessels. In contrast, dilatation of tubules is clearly observed in photomicrographs 2 and 8, along with other histopathologic abnormalities, such as basophilic changes in tubules, mineralization, and inflammatory cell infiltration (Table 1), suggesting the occurrence of histopathologic abnormalities over a wider range of the kidney in the SIADH rat model; the same symptoms may occur in human SIADH patients. Blood urea nitrogen level in the SIADH/SIADH rat model; the same symptoms may occur in human logic abnormalities over a wider range of the kidney in the filtration (Table 1), suggesting the occurrence of histopathologic changes in tubules, mineralization, and inflammatory cell infiltration in the SIADH including hyponatremia, serum hyposmolarity, oliguria, and hyperosmolarity of urine. The oliguria and hyperosmolarity of urine should overload kidney function and cause histopathologic abnormalities in the kidney due to the continuous reabsorption of water.

In conclusion, the present histopathologic studies on the kidney demonstrated the relationship between kidney abnormalities and SIADH and the therapeutic profile of VP-343 in SIADH.

REFERENCES


It is well known that vasopressin plays an important physiologic role in water metabolism, cardiovascular regulation, adrenocorticotropic hormone release, and glycogenolysis. Therefore the function of the kidney, lung, liver, adrenal gland, and heart should be overloaded in the SIADH rat model. The relative weight of the kidney, adrenal gland, and heart was significantly higher in the SIADH/control group than in the normal group, as shown in Fig. 2. Furthermore, the absolute weight of all these organs was also increased in the SIADH/control group (data not shown). These results suggest that general pathologic changes such as edema, thickening, swelling, and proliferation occur in the organs in SIADH. VP-343 normalized the relative weight of the kidney, probably by suppressing the excessive water retention in tubules via vasopressin V2 receptor antagonism. On the other hand, the absolute weight of the lung and liver, was not increased (lung, normal 1.0±0.4 g, SIADH/control 1.0±0.4 g; liver, normal 7.5±1.3 g, SIADH/control 6.3±0.3 g), suggesting less influence of vasopressin on the lung and liver than on the kidney, adrenal gland, and heart in the SIADH rat model. Furthermore, in the present study, VP-343 exhibited significant normalizing effects on the levels of serum sodium, serum osmolarity, urine volume, and urine osmolarity in the SIADH rat model, confirming the results reported previously.