OPC-41061, a Highly Potent Human Vasopressin V_{2}-Receptor Antagonist: Pharmacological Profile and Aquaretic Effect by Single and Multiple Oral Dosing in Rats

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Accepted for publication June 25, 1998 This paper is available online at http://www.jpet.org

ABSTRACT

The pharmacological profile and the acute and chronic aquaretic effects of OPC-41061, a novel nonpeptide human arginine vasopressin (AVP) V_{2}-receptor antagonist, were respectively characterized in HeLa cells expressing cloned human AVP receptors and in conscious male rats. OPC-41061 antagonized [^{3}H]-AVP binding to human V_{2}-receptors (K_{i} = 0.43 ± 0.06 nM) more potently than AVP (K_{i} = 0.78 ± 0.08 nM) or OPC-31260 (K_{i} = 9.42 ± 0.90 nM). OPC-41061 also inhibited [^{3}H]-AVP binding to human V_{1a}-receptors (K_{i} = 12.3 ± 0.8 nM) but not to human V_{1b}-receptors, indicating that OPC-41061 was 29 times more selective for V_{2}-receptors than for V_{1a}-receptors. OPC-41061 inhibited cAMP production induced by AVP with no intrinsic agonist activity. In rats, OPC-41061 inhibited [^{3}H]-AVP binding to V_{1a}-receptors (K_{i} = 325 ± 41 nM) and V_{2}-receptors (K_{i} = 1.33 ± 0.30 nM), showing higher receptor selectivity (V_{1a}/V_{2} = 244) than with human receptors. A single oral administration of OPC-41061 in rats clearly produced dose-dependent aquarexia. In treatment by multiple OPC-41061 dosing for 28 days at 1 and 10 mg/kg p.o. in rats, significant aquaretic effects were seen throughout the study period. As the result of aquarexis, hemoconcentration was seen at 4 hr postdosing although, no differences were seen in serum osmolality, sodium, creatinine and urea nitrogen concentrations at 24 hr postdosing. Furthermore, there was no difference in serum AVP concentration, pituitary AVP content or the number and affinity of AVP receptors in the kidney and liver at trough throughout the study period. These results demonstrate that OPC-41061 is a highly potent human AVP V_{2}-receptor antagonist and produces clear aquarexis after single and multiple dosing, suggesting the usefulness in the treatment of various water retaining states.

The regulation of body fluid volume is important in almost every area of medicine. AVP is well known to play a major role in water metabolism by inducing water reabsorption at the renal collecting duct through stimulation via V_{2} receptors. In various water retaining conditions, such as advanced cardiac failure (Cas et al., 1995; Goldsmith et al., 1983; Szatalowicz et al., 1981), cirrhosis (Bichet et al., 1982), nephrotic syndrome and syndrome of inappropriate ADH secretion (Barter and Schwartz, 1967; Zerbe et al., 1980), the kidneys fail to excrete the amount of water ingested, causing water retention and leading to an excessive water state. Thus, water diuretics (aquaretics) have exciting therapeutic implications in the management of patients with water excess and consequent dilutional hyponatremia (Schrier and Niederberger, 1993; Verbalis, 1993).

Although several potent peptide vasopressin antagonists are currently available (Laszlo et al., 1991; Manning and Sawyer, 1989), none has emerged as a clinically useful antidiuretic antagonist because of their low oral bioavailability, species differences and especially, antidiuretic (agonistic) effects when tested in humans (Albrightson-Winslow et al., 1981; 1991; Manning and Sawyer, 1989; Allison et al., 1988; Brooks et al., 1988; Mah and Hofbauer, 1988). Because two nonpeptide, orally effective AVP antagonists, OPC-21268 and OPC-31260, were respectively reported in 1991 and 1992 (Yamamura et al., 1992), there has been great interest in AVP research and clinical use, encouraging

Received for publication March 27, 1998.

1 This work was supported in part by research grants from Grant-in-Aid of the Japan Health Science Foundation, Tokyo, Japan.

ABBREVIATIONS: AVP, arginine vasopressin; cAMP, cyclic adenosine monophosphate; BSA, bovine serum albumin; DMEM, Dulbecco’s modified Eagle’s medium; HeLa, human endocervical carcinoma cells; V_{2}-HeLa cells, HeLa cells expressing human AVP V_{1a}-receptors; V_{1a}-HeLa cells, HeLa cells expressing human AVP V_{1a}-receptors; V_{2}-HeLa cells, HeLa cells expressing human AVP V_{2}-receptors; ANOVA, analysis of variance; CHF, congestive heart failure.
the development of various nonpeptide AVP antagonists (Freidinger and Pettibone, 1997; Serradei-Le et al., 1993, 1996; Tahara et al., 1997) and some of them have been clinically developed in patient.

The AVP receptor subtypes originally proposed by Michell et al. (1979) were based on primary intracellular signaling mechanisms: cyclic AMP-independent (V₁ₐ) and cyclic AMP-dependent (V₂) pathways. Recently, cDNAs encoding the AVP receptors have been cloned and their primary structure identified. AVP receptors were further classified into at least three subtypes: V₁a- (Morel et al., 1992; Thibonnier et al., 1994), V₁b- (De Keyzer et al., 1984; Sugimoto et al., 1994) and V₂-receptors (Birnbaumer et al., 1992; Lolait et al., 1992). Species differences between humans and rats have been reported with peptide AVP antagonists to V₂-receptors and with nonpeptide antagonists to V₃-receptors (Liu et al., 1994; Hirasawa et al., 1994; Thibonnier et al., 1993, 1994, 1996; Tahara et al., 1993, 1994, 1996; Serradeil-Le et al., 1997) and some of them have been reported to modify the development of various nonpeptide AVP antagonists (Freidinger and Pettibone, 1997; Serradei-Le et al., 1993, 1996; Tahara et al., 1997) and some of them have been clinically developed in patient.

Preparation of HeLa cells expressing human AVP receptors. The cDNAs for human vasopressin V₁a-receptor were cloned in our laboratory (Hirasawa et al., 1994). For V₁b- and V₂-receptors, cDNA synthesized from human pituitary and kidney RNA was amplified the full length of coding region by using primers for V₁a- (Sugimoto et al., 1994) and V₂- (Birnbaumer et al., 1992) receptors. Cloned cDNAs and PCR products were subcloned into pBluescript KS II (+) vector, and nucleotide sequence analysis was performed by the ABI 373A DNA Sequencer (Applied Biosystems, Inc., Foster City, CA) for both complete strands. We confirmed that the clones obtained were identical to the previously reported human V₁a- and V₂-receptor cDNA by sequencing, respectively. These cDNA fragments were ligated in SRα promoter based mammalian expression vector pME18S and resulting constructs were used for transfection.

Materials and Methods

Materials. OPC-41061 and OPC-31260 were synthesized by the Tokushima 2nd Factory of Otsuka Pharmaceutical Co., Ltd. (Tokushima, Japan). AVP was purchased from Peptide Institute Inc., Japan, lipopectamine and fetal calf serum from Gibco BRL., penicillin, streptomycin and BSA from SIGMA, DMEM from Nissui Pharmaceutical Co., ³H-AVP from New England Nuclear, AVP RIA kit from Mitsubishi Petrochemical Co., and G418 (Geneticin disulfate) and other chemicals from Wako Pure Chemicals, Japan.

Animals. Male Sprague-Dawley rats (SD rats) were purchased from Charles River Japan, Inc., at 6 or 7 wk of age and housed during the experiment in an air-conditioned (temperature-, humidity- and light-controlled) animal room with free access to food and water. All experiments were performed under the regulations of the Guideline for Animal Experimentation in Otsuka Pharmaceutical Co., Ltd.

Preparation of HeLa cells expressing human AVP receptors. The cDNAs for human vasopressin V₁a-receptor was cloned in our laboratory (Hirasawa et al., 1994). For V₁b- and V₂-receptors, cDNA synthesized from human pituitary and kidney RNA was amplified the full length of coding region by using primers for V₁a- (Sugimoto et al., 1994) and V₂- (Birnbaumer et al., 1992) receptors. Cloned cDNAs and PCR products were subcloned into pBluescript KS II (+) vector, and nucleotide sequence analysis was performed by the ABI 373A DNA Sequencer (Applied Biosystems, Inc., Foster City, CA) for both complete strands. We confirmed that the clones obtained were identical to the previously reported human V₁a- and V₂-receptor cDNA by sequencing, respectively. These cDNA fragments were ligated in SRα promoter based mammalian expression vector pME18S and resulting constructs were used for transfection.

HeLa cells, grown in 5% CO₂ at 37°C, were plated at a density of 1 × 10⁶ cells/10-cm plate in 10 ml of DMEM supplemented with 10% fetal calf serum and penicillin/streptomycin, and allowed to attach overnight. Each expression vector for vasopressin receptors and pSV2neo vector (Clontech), which contained neomycin resistance gene for selection of stable transformants, were cotransfected into HeLa cells by lipofectamine method (Harwely-Nelson et al., 1989). After overnight transfection, cells were fed with fresh medium, allowed to grow for 2 days, adjusted to a density of 1 × 10⁶ cells/plate, and incubated for an additional 24 hr. Cells were continually selected for 3 to 4 wk in medium containing 400 to 800 μg/ml of the antibiotic G418. Single colonies were then isolated, expanded and harvested for radioligand binding assays to measure expression of receptors. Cells were maintained in the medium containing 200 μg/ml of G418. The medium was changed every 3 days, and the cells were subcultured after trypsinization.

Preparation of rat liver and kidney plasma membranes. Liver and kidney plasma membranes were prepared from SD rats (300–400 g, Charles River, Yokohama, Japan). Details of the methods used have been published previously (Yamamura et al., 1991, 1992). Protein concentration was measured by the method of Bradford using BSA as a standard.

Radioligand binding assay to HeLa cells. After reaching confluence in 12-well (V₁a-, V₁b-HeLa) or 24-well (V₂-HeLa) dishes, the cells were washed twice with ice-cold PBS and incubated for 2 hr with [³H]-AVP at 4°C in DMEM medium adjusted to pH 7.4 using 10 mM HEPES-NaOH containing 0.3% BSA. For the competition experiment, OPC-41061 and OPC-31260 were dissolved in dimethyl sulfoxide, diluted with DMEM medium and added into the wells at several appropriate concentrations. After 2 hr of incubation, the cells were rinsed twice with ice-cold phosphate-buffered saline, lysed in 500 (12-well) or 250 (24-well) μl of 0.1 N NaOH containing 0.1% sodium dodecyl sulfate, transferred into scintillation vials, combined with 5 ml of Aquazol II (NEN, Boston, MA) and detected using a liquid scintillation counter (LSC-1050, Aloka, Tokyo, Japan). The protein content of the lysed cells was determined by the method of Bradford.

Radioligand binding assay to rat kidney and liver plasma membranes. Binding assay to rat kidney and liver plasma membranes was performed as described previously (Yamamura et al., 1991, 1992).

cAMP production in V₂-HeLa by AVP. After reaching confluence in 24-well dishes, the V₂-HeLa cells were washed twice with ice-cold PBS and incubated for 10 min with AVP at 1 nM in the absence or presence of OPC-41061 at 37°C in DMEM medium adjusted to pH 7.4 using 10 mM HEPES-NaOH containing 1 mM 3-isobutyl-1-methylxantine and 0.3% BSA. For the competition ex-
periment, OPC-41061 and OPC-31260 were dissolved in DMSO, diluted with DMEM medium and added into the wells at several appropriate concentrations. After 10 min of incubation, the medium was evacuated and the cells were rinsed once with ice-cold PBS. cAMP was extracted from the cells using 250 μl of 0.1 N HCl solution and stored at −20°C until determination using a radioimmunoassay kit (Yamasa, Tokyo, Japan).

**Single-dosing experiments in rats.** OPC-41061 suspended in 1% hydroxyethyl cellulose or the solvent was administered orally at doses of 0.3 to 10 mg/kg to rats at 8 wk of age (body weight: 280–340 g) by a gastric tube. Seven animals in each dosing group were then placed individually in metabolic cages and spontaneously voided urine was collected for the periods of 0 to 2, 2 to 4, 4 to 6, 6 to 8 and 8 to 24 hr. Another five animals in each group were decapitated at 4 hr postdosing and trunk blood was collected. After centrifugation at 3000 rpm for 10 min, the serum was obtained and frozen until use.

Osmolality was determined by freezing point depression using a Fiske osmometer (Model 3400, Boston, MA). Electrolytes were measured by the ion-electrode method (CX-3, Beckman Instruments, Fullerton, CA), and creatinine and urea nitrogen were measured using a COBAS autoanalyzer (COBAS FARAII, Roche, Basel, Switzerland). AVP was measured by radioimmunoassay using a AVP-RIA kit (Mitsubishi Petrochemical Co. Ltd., Tokyo, Japan).

**Multiple-dosing experiments in rats.** Male SD rats at 7 wk of age (body weight: about 260 g) were divided into two groups. One group (group A) were periodically examined for aquarectic effect and the other (group B) were decapitated for determination of AVP-receptors and AVP content. Six animals were used in each group. OPC-41061 was suspended in 1% hydroxyethyl cellulose solution and orally administered at doses of 1 and 10 mg/kg once daily at around 9:30 A.M. The rats in group A were placed individually in metabolic cages and spontaneously voided urine was periodically (day −1, 1, 3, 7, 10, 14, 21 and 28) collected for the periods of 0 to 4 and 4 to 24 hr. The rats in group B were decapitated at 24 hr postdosing on days 7, 14 and 21 and those in group A were decapitated at 24 hr postdosing on day 28. In the control group, rats were decapitated at predosing (day 1) to obtain basal values. The kidney and liver (for analysis of AVP receptors) and the pituitary (for determination of AVP content) were quickly removed and immediately cooled with ice-cold saline. The removed pituitary was weighed and then stored frozen at −80°C until extraction.

Kidney and liver plasma membranes of each rat were prepared as described above. The Kd and Bmax were determined by Scatchard analysis of [3H]-AVP saturation binding. The pituitary was combined with 1 ml of 0.1 N HCl, sonicated for 30 sec using a sonicator (Microson, output: 6), and then centrifuged. The supernatant was diluted 500- to 5000-fold with saline containing 0.1% BSA. AVP content was determined by radioimmunoassay using an AVP-RIA kit.

**Binding data analysis.** The dissociation constant (Kd) and the number of binding sites (Bmax) were determined by Scatchard analysis of [3H]-AVP saturation binding. The IC50 values of test compounds were determined by displacement experiments. The inhibition constant (Kd) was calculated from the IC50 values using the Cheng and Prusoff equation (1973).

**In vivo data analysis.** Data from the single-dosing experiment were analyzed by one-way analysis of variance (ANOVA) followed by two-tailed Dunnett’s test at a significance level of P < .05. Data from the repeated-dosing experiment were analyzed by ANOVA based on repeated measurements. When ANOVA indicated statistical significance, differences were analyzed by two-tailed Dunnett’s multiple-comparison test at each measurement day. Differences were considered significant at P < .05.

**Results**

**Antagonistic affinities of OPC-41061 for AVP receptors.** As shown in table 1, HeLa cells transfected with each subtype of human AVP receptors constantly expressed a sufficient number of receptors for [3H]-AVP binding assay through repeated passaging. In V2-HeLa cells, the expressed V2-receptors acted functionally and stimulated adenylate cyclase after stimulation by AVP (fig. 2). AVP, even at the minimum concentration of 10−12 M, increased the production of cAMP (281 ± 98% from the basal value), with the maximum increase achieved at 10−8 M.

OPC-41061 and OPC-31260 displaced [3H]-AVP binding to human V2-receptors expressed on HeLa cells in a concentration-dependent manner, and the inhibition curve paralleled the curve for AVP (fig. 3). The Kd value for OPC-41061 was 1.8 times higher than that for AVP and 22 times higher than that for OPC-31260, indicating that OPC-41061 had the most potent affinity for human V2-receptors. Both compounds also inhibited [3H]-AVP binding to V1a-receptors, but not to human V1b-receptors (table 2). In comparison with the affinity to rat AVP receptors, OPC-41061 showed 3 times more potent antagonism of V2-receptors and 26 times more potent antagonism of V1a-receptors in humans than in rats, indicating that OPC-41061 is a more potent antagonist of human AVP receptors than rat AVP receptors. However, OPC-31260 showed almost same affinity to both human and rat V2 receptors. OPC-41061 was 29 times more selective to human V2-receptors than to human V1b-receptors and 244 times more selective to rat V2-receptors than that to rat V1a-receptors.

Furthermore, to confirm the antagonistic activity, we examined the effect of OPC-41061 on adenylate cyclase activity induced by AVP in V2-HeLa cells (fig. 2). At the submaximum dose of 10−8 M, AVP increased cAMP generation by 1520% from the control. OPC-41061 dose-dependently inhibited the increase of cAMP production by 1 nM of AVP. However, OPC-41061 alone did not increase cAMP production at up to 10−6 M (data not shown).

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characterization of human AVP receptors expressed on cultured HeLa cells for [3H]-AVP binding</th>
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<tbody>
<tr>
<td></td>
<td>V2</td>
</tr>
<tr>
<td>Kd (nM)</td>
<td>1.09 ± 0.05</td>
</tr>
<tr>
<td>Bmax (fmol/mg protein)</td>
<td>1021 ± 98</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M. of at least four separate determinations performed in duplicate.
These data clearly show that OPC-41061 possesses a more potent affinity for human V₂-receptors than native AVP and that OPC-41061 can clearly antagonize V₂-receptors with no intrinsic agonistic activity.

Aquaretic effect by single-dosing in conscious rats. A single oral administration of OPC-41061 increased urine volume and decreased urine osmolality in a dose-dependent manner at doses of 0.3 to 10 mg/kg in normally hydrated conscious rats (fig. 4). The maximum urine output for 2 hr postdosing was 18.0 ± 2.6 ml, which was 12 times higher than the control, and urine osmolality reached a minimum of 175 ± 15 mOsm/kg (vs. 714 ± 136 mOsm/kg for the control). No significant increase in urine output was seen after 4 hr postdosing (data not shown). Urinary Na excretion during the 4 hr postdosing increased dose-dependently. However, the magnitude of the increase was considerably smaller than that by natriuretic agents such as furosemide [data shown previously (Yamamura et al., 1992)].

At 4 hr postdosing, serum osmolality and Na and creatinine concentrations were significantly elevated presumably by the decreased body fluid resulting from the aquaretics induced by OPC-41061 (table 3). Serum AVP concentration also increased dose-dependently after the elevation of serum osmolality. In spite of the hemoconcentrated status at 4 hr postdosing, blood urea nitrogen was dose-dependently decreased presumably by OPC-41061 inhibition of urea reabsorption at the terminal of the inner medullary collecting duct, leading to an increase in urinary urea excretion.

Aquaretic effect by multiple-dosing in conscious rats. OPC-41061 was orally administered once daily at 1 and 10 mg/kg/day for 28 days. No differences in body weight gain (fig. 5). Figure 6 and table 4 show the effects on urine excretion and serum parameters in rats after multiple dosing of OPC-41061. Throughout the experiment, OPC-41061 at doses of 1 and 10 mg/kg significantly increased urine volume collected during 0 to 4 hr postdosing and decreased urine osmolality. At 1 mg/kg urine volume remained constant throughout the study, but at 10 mg/kg it gradually decreased. However, urine osmolality remained constant at both doses and at each level showed significant differences compared with the control, suggesting that 4-wk repeated administration of OPC-41061 did not alter the compound’s aquaretic effect. The excretion of Na and urea nitrogen in the urine collected during 0 to 4 hr postdosing were significantly increased in the OPC-41061 groups, but there were no differences in the net excretion collected during 0 to 24 hr postdosing. There were no differences in the net urinary excretion of creatinine between the OPC-41061 groups and the control group at day 7 was 150 ± 160 fmol/mg, respectively, and 122 ± 12 fmol/mg at day 28, indicating that OPC-41061 did not affect glomerular filtration rate. Urinary AVP excretion was significantly increased in the urine collected during 0–4 hr and 0–24 hr postdosing and remained almost constant throughout the study. It is important to note that, in addition to the constant change in urine osmolality, the constant AVP excretion during the study period further support the conclusion that repeated administration did not alter the aquaretic effect of OPC-41061.

Although serum osmolality and Na, blood urea nitrogen and AVP concentrations were significantly changed at 4 hr postdosing (table 3), no differences were observed in those parameters at 24 hr postdosing and the 24-hr values remained at the control level throughout the experiment. The maximum binding capacity (Bₘₐₓ) of V₂-receptors (kidney) of 1 and 10 mg/kg/group at day 7 was 150 ± 15 and 160 ± 23 fmol/mg, respectively, although control group was 166 ± 17 fmol/mg. The value at day 28 was 109 ± 6 fmol/mg and 126 ± 7 fmol/mg for 1 and 10 mg/kg, respectively, and 122 ± 12 fmol/mg.

TABLE 2
Antagonistic potency of OPC-41061 and OPC-31260 for human and rat AVP receptors

<table>
<thead>
<tr>
<th></th>
<th>Human V₂</th>
<th>V₁a</th>
<th>V₁b</th>
<th>Rat V₂</th>
<th>V₁a</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVP</td>
<td>0.78 ± 0.08</td>
<td>0.84 ± 0.08</td>
<td>0.59 ± 0.05</td>
<td>0.95 ± 0.31</td>
<td>1.45 ± 0.26</td>
</tr>
<tr>
<td>OPC-41061</td>
<td>0.43 ± 0.06</td>
<td>12.3 ± 0.8</td>
<td>&gt;100,000</td>
<td>1.33 ± 0.30</td>
<td>325 ± 41</td>
</tr>
<tr>
<td>OPC-31260</td>
<td>9.42 ± 0.90</td>
<td>150 ± 15</td>
<td>&gt;100,000</td>
<td>6.36 ± 1.56</td>
<td>524 ± 119</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M. of at least four separate determinations performed in duplicate.
fnmol/mg for control. The dissociation constant ($K_d$) of control, 1 and 10 mg/kg at day 7 was 1.94 ± 0.19, 1.73 ± 0.30 and 1.70 ± 0.42 nM and at day 28 was 1.10 ± 0.05, 1.22 ± 0.13 and 1.23 ± 0.10 nM, respectively. The $B_{max}$ and $K_d$ values of $V_2$-receptors (liver) also did not change at trough throughout the experiment. Furthermore, pituitary AVP content was constant throughout the experiment (data not shown).

These results suggested that although oral administration of OPC-41061 increased AVP secretion from the pituitary, the secretion recovered to the basal level by 24 hr postdosing. In contrast, serum renin activity was gradually decreased and serum aldosterone concentration was significantly lowered through repeated administration of OPC-41061 for 28 days (table 5).

In conclusion, orally administered OPC-41061 is a powerful aquaretic, and the aquaretic effect was unchanged during repeated administration for 4 wk.

**Discussion**

Aquaretics have exciting therapeutic implications in the management of patients with water excess and consequent dilutional hyponatremia, as in patients with congestive heart failure and cirrhosis, or in patients with euvolemic hyponatremia, as in patients with syndrome of inappropriate ADH secretion. There is an obvious need for a potent $V_2$-receptor antagonist that can be safely administered orally over the long term in a clinical setting. Previous reports of chronic blockade of vasopressin receptors by a peptide $V_2$-antagonist did not show persistent aquarexis (Hofbauer et al., 1988; Mah et al., 1988). A peptide $V_2$-antagonist, d-(CH$_2$)$_2$-D-Tyr($\varepsilon$)EtVAVP, increased urine volume and decreased urine osmolality after i.v. or s.c. administration in normally hydrated or dehydrated Sprague-Dawley rats in acute experiments. However, by chronic administration by i.v. infusion or repeated s.c. dosing, water excretion and intake increased markedly on the first day but then subsequently reverted to normal, although the marked initial water loss was fully compensated for by an increased water intake. The lack of a chronic effect with peptide antagonists is thought to be due to the activation of endogenous compensatory mechanisms such as hormones, prostaglandins and renal and systemic hemodynamics. Alternatively, the lack of effect may be due to an intrinsic agonism which might have contributed to the normalization of water balance by limiting the maximum antidiuretic effects of renal tubular AVP receptors.

OPC-41061 did not increase cyclic-AMP production in $V_2$-HeLa cells and did not show any antidiuretic effect in water-loaded, alcohol-anesthetized rats (data not shown), indicating that OPC-41061 possesses no agonistic activity for $V_2$-receptors. Therefore, OPC-41061 is expected to produce a chronic aquaretic effect during multiple dosing. In this report, we examined changes not only in the aquaretic effect of

**Table 3**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Osmolality (mOsm/kg)</th>
<th>Na (mEq/l)</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>AVP (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>293 ± 2</td>
<td>145 ± 0</td>
<td>17.1 ± 0.7</td>
<td>0.58 ± 0.01</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>294 ± 1</td>
<td>147 ± 1</td>
<td>14.7 ± 0.6*</td>
<td>0.60 ± 0.02</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>294 ± 2</td>
<td>148 ± 1*</td>
<td>12.0 ± 0.4*</td>
<td>0.58 ± 0.01</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>301 ± 3*</td>
<td>152 ± 1*</td>
<td>12.6 ± 0.5*</td>
<td>0.71 ± 0.02*</td>
<td>4.4 ± 0.9</td>
</tr>
</tbody>
</table>

*Values are expressed as the mean ± S.E.M. (n = 5). Differences between the OPC-41061 groups and the control group were analyzed by one-way ANOVA followed by two-tailed Dunnett’s test (*P < .05, *P < .01 vs. the control). Differences in serum AVP levels were not statistically analyzed, because the control value was below the detection limit.

**Fig. 5.** Effects of multiple dosing of OPC-41061 on body weight in male SD rats. Values are expressed as the mean ± S.E.M. (n = 6). Differences between the OPC-41061 groups and the control group were analyzed by ANOVA based on repeated measurements, but no statistical significance was seen.

**Fig. 6.** Effects of multiple dosing of OPC-41061 on urine collected during 0 to 4 hr postdosing in normally hydrated male SD rats. Values are expressed as the mean ± S.E.M. (n = 6). Differences between the OPC-41061 groups and the control group were analyzed by ANOVA based on repeated measurements (**P < .01) and any significant differences were then analyzed by two-tailed Dunnett’s multiple-comparison test at each measurement day (*P < .05, **P < .01 vs. the control).
TABLE 4

Effects of multiple dosing of OPC-41061 on serum parameters at 24-hr postdosing in male SD rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Osmolality (mOsm/kg)</th>
<th>Na (mEq/liter)</th>
<th>Creatinine (mg/dl)</th>
<th>Urea nitrogen (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg</td>
<td>305</td>
<td>142.1</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>10 mg</td>
<td>308</td>
<td>142.4</td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M. (n = 6). Differences between the OPC-41061 groups and the control group were analyzed by ANOVA based on repeated measurements and any significant differences were then analyzed by a two-tailed Student’s multiple-comparison test at each measurement day (P < .05 vs. the control).

OPC-41061 but also in the circulating AVP system and targeted organ function by chronic oral administration of OPC-41061 for 28 days in rats. Urine volume did not change throughout the study period in the 1-mg/kg group, but it decreased slightly in the 10-mg/kg group. The slight decrease in urine volume was considered to be the result of activated compensatory mechanisms. There have been similar reports that the natriuresis by chronic hydrochlorothiazide or furosemide administration markedly abated and sodium excretion was returned to the control level in a so-called “braking effect” (Kahn et al., 1983; Walter and Shirley, 1986). However, in our study, urine volume was decreased only slightly in the first few days and did not return to the control level as seen in the experiments with peptide AVP antagonists and with hydrochlorothiazide and furosemide. Furthermore, urine osmolality and urinary AVP excretion showed constant change throughout the study period, indicating that the aquaretic effect of OPC-41061 was unchanged during the 28 days of repeated administration. Although OPC-41061 increased the excretion of Na and urea nitrogen in the urine collected during 0 to 4 hr postdosing, serum Na concentration was increased and serum urea nitrogen was decreased significantly at 4 hr postdosing. This discrepancy seems attributable to the fact that hemoconcentration by the increased free water clearance exceeds the Na excretion but not the urea excretion. These changes then returned to the control levels at 24 hr postdosing, because there were no differences in the urine collected during 0 to 24 hr postdosing between the treated and control groups. The excretion of AVP was increased during 0 to 4 hr postdosing and remained almost the same throughout the study period. Serum AVP concentration was increased at 4 hr postdosing and subsequently returned to the control values. There were also no differences in serum AVP concentration, pituitary AVP level, or the number and dissociation constant of AVP receptors in the liver and kidney between the treated and control groups. Although oral administration of OPC-41061 increased AVP secretion from the pituitary and increased circulating AVP for several hours after dosing, both parameters recovered to the basal level by 24 hr postdosing. Because the marked aquarexia by OPC-41061 did not last past 4 hr postdosing, and the initial water loss was fully compensated for by an increasing water intake, there seemed to be no significant changes in the AVP producing and secreting organ and the AVP target receptors. In other circulating hormone systems, serum renin activity tended to be decreased and aldosterone secretion was significantly decreased following by chronic administration. The suppression of renin and aldosterone secretion might be caused by a direct action of the increased AVP on the juxtaglomerular apparatus (Reid et al., 1983) or secondarily to an apparent increases in serum sodium after OPC-41061 dosing.

Congestive heart failure is a complex clinical syndrome characterized by a number of neuroendocrine responses. Recent work has established the importance of the renin-angiotensin systems and angiotensin-converting enzyme inhibitors have emerged as distinctly useful drugs in CHF. Therefore, it may be desirable for drugs used in the treatment of CHF to not activate the renin-angiotensin-aldosterone system. Diuretic therapy is a mainstay in the treatment of edematous conditions in CHF. The conventional diuretics (natriuretics) now in use are effective for the treatment of...
edematous states, but they are associated with activation of the renin-angiotensin-aldosterone systems. Furthermore, natriuretics may cause electrolyte imbalance, such as hyponatremia or hypokalemia. Serum sodium concentration is one of the most powerful predictors of cardiovascular mortality, with hyponatremia patients showing substantially shorter survival than patients with a normal serum sodium concentration (Lee and Packer, 1986). It is not known whether neurohormonal activation and decreased serum Na level are the results of a more advanced degree of heart failure or whether they contribute directly to the progression of mortality. As a result of the aquaresis demonstrated in this report, aquaretics have a potential medical benefit for the treatment of edematous conditions in CHF by removing excess water from the body without activating the renin-angiotensin-aldosterone system or causing serum electrolyte imbalances.

In conclusion, OPC-41061 was characterized as a highly potent nonpeptide human AVP V$_2$-receptor antagonist with no agonistic properties. The compound's aquaretic effects by single administration were confirmed by an increase in urine volume and a decrease in urine osmolality followed by an increase in serum sodium concentration. Chronic administration for 28 days also confirmed a sustained aquaretic effect with no change in pituitary AVP content or target AVP receptors. Thus, these results support the contention that OPC-41061 may be therapeutically useful for the treatment of various diseases involving fluid body retention. It is also expected that aquaretics will be well-suited for chronic therapy without the well-known side effects of conventional diuretics (natriuretics) on the balance of electrolytes, such as hyponatremia or hypokalemia, and that aquaretics may make possible optimum diuretic therapy in water-retaining states.

Acknowledgments

The authors thank Dr. Y. Yabuuchi for his valuable comments and continuing encouragement and to Dr. M. Thornton for comments on this manuscript. We also thank Ms. M. Murakami for her excellent technical assistance and Ms. M. Asano and Ms. Y. Matsuuchi for their excellent assistance in the preparation of this manuscript.

References


### Table 5

<table>
<thead>
<tr>
<th>Day</th>
<th>AVP (pg/ml)</th>
<th>Renin Activity (ng/ml/hr)</th>
<th>Aldosterone (pg/ml)</th>
<th>Pituitary AVP Content (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control 1 mg</td>
<td>10 mg</td>
<td>Control 1 mg</td>
<td>10 mg</td>
</tr>
<tr>
<td>0</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.0</td>
<td>10.0 ± 1.1</td>
<td>0.3 ± 0.0</td>
</tr>
<tr>
<td>7</td>
<td>0.2 ± 0.1</td>
<td>0.2 ± 0.0</td>
<td>10.0 ± 1.1</td>
<td>0.2 ± 0.0</td>
</tr>
<tr>
<td>14</td>
<td>0.4 ± 0.0</td>
<td>0.6 ± 0.2</td>
<td>10.0 ± 1.1</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>21</td>
<td>0.6 ± 0.1</td>
<td>1.8 ± 1.0</td>
<td>10.0 ± 1.1</td>
<td>1.8 ± 1.0</td>
</tr>
<tr>
<td>28</td>
<td>2.1 ± 0.3</td>
<td>0.4 ± 0.0</td>
<td>10.0 ± 1.1</td>
<td>0.4 ± 0.0</td>
</tr>
</tbody>
</table>

Values are expressed as the mean ± S.E.M. (n = 6). Differences between the OPC-41061 groups and the control group were analyzed by ANOVA based on repeated measurements and any significant differences were then analyzed by two-tailed Dunnett's multiple-comparison test at each measurement day (*P < .05, **P < .01 vs. the control).


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