Antidiuretic Effects of a Novel Nonpeptide Vasopressin V2-Receptor Agonist, OPC-51803, Administered Orally to Dogs

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Abstract. We elucidated the pharmacological properties of a novel nonpeptide vasopressin V2-receptor agonist, OPC-51803 ((5R)-2-[1-(2-chloro-4-(1-pyrrolidinyl)benzoyl-2,3,4,5-tetrahydro-1H-1-benzazepine-5-yl]-N-isopropylacetamide), via both in vitro binding experiments incorporating canine kidney and platelet membrane fractions and in vivo experiments that would determine the compound’s antidiuretic effects after oral administration to water-loaded dogs. OPC-51803 displaced [3H]arginine vasopressin (AVP) binding to canine V2 and V1a receptors, as determined by resulting Ki values of 15.2 ± 0.6 nM (n = 4) and 653 ± 146 nM (n = 4), respectively. These data indicate that OPC-51803 was about 43 times more selective for V2 receptors than for V1a receptors. Antidiuretic studies showed that orally administered doses of OPC-51803 (0.03 to 0.3 mg·kg⁻¹) decreased urine volume and increased urinary osmolality in a dose-dependent manner in water-loaded dogs. Intravenous OPC-51803 infusions (0.3 and 3 µg·kg⁻¹·min⁻¹) did not affect renal or systemic hemodynamics in anesthetized dogs. Since these results confirm that OPC-51803 shows antidiuretic action in dogs, the compound may be useful for treating AVP-deficient pathophysiological states.

Keywords: OPC-51803, nonpeptide, V2 agonist, antidiuresis

Introduction

Arginine vasopressin (AVP) is a peptide hormone that increases water permeability through the V2 receptor at renal collecting ducts. Antidiuretic action is caused from a rise in cyclic adenosine 3',5'-monophosphate (cAMP) production and water channel transfer (aquaporin-2) from stores in intracellular vesicles to the apical plasma membrane. Hence, AVP plays an important role in maintaining water homeostasis.

Recently, we discovered a nonpeptide AVP V2-receptor agonist, OPC-51803 (1, 2), by the lead optimization of congeners found in the nonpeptide V2-receptor antagonists OPC-31260 (3, 4) and OPC-41061 (5–7). In vitro binding experiments, OPC-51803 selectively bound to V2 receptors in human AVP receptor subtype-expressing HeLa cells and rat plasma membranes. In addition, in vivo experiments showed that orally administered OPC-51803 significantly decreased urine volume and increased urinary osmolality in both Brattleboro rats (have a hereditary AVP deficiency) and normally hydrated rats. Although the results show that OPC-51803 is a potent antidiuretic in rats, the compound’s effects in dogs have not been elucidated.

In previous reports, AVP analogs, which are either peptide or nonpeptide compounds, showed a difference between animal species (8–11). In particular, peptide V2-receptor antagonists (e.g., SK&F 101926) showed potent diuresis by inhibiting the action of endogenous AVP in rats. In water-loaded dogs, however, it had the opposite effect (8, 9). Here, we estimated OPC-51803’s affinity for canine V2 receptors and its agonistic activities in water-loaded dogs and also determined the pharmacological profile of OPC-51803 (including renal hemodynamics).
Materials and Methods

Materials

OPC-51803, (5R)-2-[(2-chloro-4-(1-pyrrolidinyl)benzoyl)-2,3,4,5-tetrahydro-1H-1-benzazepin-5-yl]-N-isopropylacetamide, was synthesized at Otsuka Pharmaceutical Co., Ltd. (Tokyo). AVP was purchased from Peptide Institute, Inc. (Osaka), and [3H]AVP was purchased from NEN™ Lifescience Products, Inc. (Boston, MA, USA). 1-Desamino-8-D-arginine vasopressin (DDAVP) and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO, USA). Phosphate-buffered salts were purchased from Takara Shuzo Co., Ltd. (Kusatsu). Ethylenediaminetetraacetic acid (EDTA) was purchased from Dojindo Laboratories (Kumamoto), and dextran T500 (DEX) was purchased from Pharmacia Biotech AB (Uppsala, Sweden). Polyethylene glycol 6000 (PEG) and other reagents were purchased from Wako Pure Industries, Inc. (Osaka).

Animals

Male beagle dogs (9.3 – 10.9 kg) and mongrel dogs (12 – 22 kg) were purchased from Nihon Nosan Kogyo (Yokohama) and Kitayama Labes Co., Ltd. (Nagano), respectively. The dogs were housed in a temperature-, humidity-, and light-controlled room and given 300 to 400 g per day of food (CD-5; Clea Japan, Inc., Tokyo) along with free access to water. The care and handling of these animals were in accordance with “Guidelines for Animal Care and Use in Otsuka Pharmaceutical Co., Ltd.,” October 1, 1994.

Membrane preparations of canine platelet and kidney medulla

Crude platelet membrane fractions were prepared immediately before each binding experiment at 1-week intervals by modifying previously described methods (12, 13). Briefly, blood was obtained from the cephalic vein of six conscious beagle dogs. The blood was anticoagulated with 3.8% sodium citrate (1:9 v:v) and centrifuged at 200 x g for 15 min at room temperature. The supernatant (platelet-rich plasma) was centrifuged at 2000 x g for 20 min at 4°C, and the pellets were resuspended by the PEG-DEX double-phase system to obtain a membrane fraction at an interfacial phase. The membrane fraction was washed twice with phosphate-buffered saline (PBS), suspended in 50% glycerol-PBS, and stored at -80°C until use. A sample of each preparation was dissolved in 0.1 N NaOH, and the resulting protein was quantitated by the dye method (14) using BSA as a standard.

Radioligand binding assay of canine platelet and kidney membrane fractions

For the saturation binding studies, each membrane preparation was incubated with [3H]AVP at various concentrations. Non-specific binding was determined in the presence of 1 µM unlabeled AVP. In the competition studies, multiple concentrations of OPC-51803, AVP, and DDAVP were incubated with 6 nM of [3H]AVP and platelet membranes or 3 nM of [3H]AVP and kidney membranes. Samples were incubated for 10 min at 37°C (platelet membranes) or 20 min at 25°C (kidney membranes). In preliminary studies we confirmed that the preparations achieved equilibrium under these conditions (data not shown).

Radioligand binding data analysis

The dissociation constant (Kd) and the number of binding sites (Bmax) were determined by Scatchard analysis (15). The IC50 values of OPC-51803, AVP, and DDAVP were determined by a displacement procedure using [3H]AVP binding, and the resultant curves were resolved to obtain the concentrations required for a 50% inhibition of specific binding. The inhibition constants (Ki) were calculated from the IC50 values using the Cheng and Prusoff equation (16).

Antidiuretic action of OPC-51803 in water-loaded dogs

To induce water diuresis, 50 ml·kg⁻¹ of tap water was administered to beagle dogs using a stomach tube. After physically restraining the dogs with loose slings, one indwelling catheter was inserted in the cephalic vein to infuse a hypotonic solution (3% glucose solution) and once was inserted in the bladder to gather urine samples. The intravenous infusion rate of a hypotonic solution was initially about 5 ml·min⁻¹ and equal to the rate of urine excretion after urine volume became

All kidneys were isolated, and the cortex and adipose tissues were removed to leave as much of the medulla as possible. The medulla was minced with scissors and homogenated using a Polytron® (setting 6, 15 s, 2 times; Kinematica AG, Lucerne, Switzerland) in a 0.25 M sucrose buffer (pH 7.4) containing 5 mM MgCl₂, 1 mM EDTA, and 10 mM Tris. The suspension was centrifuged at 2000 x g for 15 min at 4°C, then the pellets were resuspended by the PEG-DEX double-phase system to obtain a membrane fraction at an interfacial phase. The membrane fraction was washed twice with phosphate-buffered saline (PBS), suspended in 50% glycerol-PBS, and stored at -80°C until use. A sample of each preparation was dissolved in 0.1 N NaOH, and the resulting protein was quantitated by the dye method (14) using BSA as a standard.

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Effects of OPC-51803 on hemodynamics in anesthetized dogs

Mongrel dogs were anesthetized with a bolus injection (30 mg·kg⁻¹, i.v.) and a subsequent infusion (5 mg·kg⁻¹·h⁻¹) of sodium pentobarbital, intubated, and artificially ventilated with room air using a respirator (Model SN-480-3; Shinano, Tokyo). The cephalic vein was cannulated for drug administration. The brachial artery was cannulated to collect arterial blood samples; mean arterial pressure (MAP) and heart rate (HR) were measured with a pressure transducer. A catheter was inserted into the left ureter to collect urine. The left kidney was exposed by a retroperitoneal flank incision, and an electromagnetic flow probe (2.5 – 3.5 mm in diameter; Nihon Kohden, Tokyo) was attached to the renal artery to measure renal blood flow (RBF) with a square-wave flowmeter (MFV-2100, Nihon Kohden). Inulin, dissolved in a hypotonic solution (0.45% NaCl and 2.5% dextrose), was given intravenously at an initial dose of 50 mg·kg⁻¹ and infused at a dose of 1 mg·kg⁻¹·min⁻¹ (0.1 ml·kg⁻¹·min⁻¹) to measure inulin clearance, which is an index of glomerular filtration rate (GFR). After surgery, the animals were allowed to rest for 60 to 180 min to allow urine volume and hemodynamic parameters to stabilize.

When urine was constantly excreted, samples were collected over 20-min periods to measure two consecutive basal values. Then, OPC-51803 (0.3 and 3.0 μg·kg⁻¹·min⁻¹) or 20% dimethylformamide (vehicle) was intravenously infused at a rate of 0.2 ml·min⁻¹ over a 60-min period, and urine was collected for three consecutive 20-min periods. At the midpoint of each period, arterial blood samples were drawn and MAP, HR, and RBF were recorded on a polygraph system (Recti-horiz-8K; NEC-San-ei, Tokyo). GFR was calculated as follows:

\[
\text{GFR} = \text{urine flow rate (ml·min}^{-1}) \times \frac{\text{U}_{\text{inulin}}}{\text{P}_{\text{inulin}}},
\]

where \( U_{\text{inulin}} \) is urinary inulin concentration, and \( P_{\text{inulin}} \) is plasma inulin concentration. The inulin concentrations were measured by Anthrone methods (Spectrophotometer, Spectra Max 250; Wako) (17). Urinary and plasma osmolality were determined using the Fiske Osmometer; and sodium, potassium, and urea nitrogen concentrations were measured by ion-selective potentiometry and an enzymatic-conductivity rate method using an autoanalyzer (Synchron CX-3; Beckman Instruments, Brea, CA, USA). Plasma AVP concentrations were measured by radioimmunoassay (Mitsubishi Chemical, Tokyo).

Analysis of in vivo data

All values were expressed as means ± S.E.M. Urine flow, GFR, RBF, and urinary excretions of sodium, potassium, and urea nitrogen were divided by the body weight of each dog. The differences between the vehicle-treated and OPC-51803-treated groups for urine, plasma, and hemodynamic parameters were determined by repeated measures ANOVA. When either the effects of a group or group-time interaction were significant, the difference at each time point was analyzed by a two-tailed Dunnett’s multiple comparison test. The differences were considered significant at \( P<0.05 \).

Results


When [^3]H]AVP concentrations were increased, saturation binding occurred. The \( K_d \) and \( B_{\text{max}} \) values calculated from a Scatchard analysis were 0.69 ± 0.08 nM and 236 ± 8 fmol·mg⁻¹ protein for canine V₂ receptors (n = 4) and 2.02 ± 0.16 nM and 40 ± 4 fmol·mg⁻¹ protein for canine V₄a receptors (n = 4), respectively. In competition binding experiments, OPC-51803 displaced [^3]H]AVP binding to canine V₂ and V₄a receptors in a

<p>| Table 1. Inhibition constants (( K_i )) of OPC-51803, AVP, and DDAVP for canine V₂ and V₄a receptors |
|---------------------------------------------------------|------------------------|------------------------|------------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Kidney (V₂ receptors)</th>
<th>Platelet (V₄a receptors)</th>
<th>Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>OPC-51803</td>
<td>15.2 ± 0.6</td>
<td>653 ± 146</td>
<td>43</td>
</tr>
<tr>
<td>AVP</td>
<td>0.62 ± 0.06</td>
<td>1.47 ± 0.19</td>
<td>2.4</td>
</tr>
<tr>
<td>DDAVP</td>
<td>0.59 ± 0.02</td>
<td>772 ± 213</td>
<td>1308</td>
</tr>
</tbody>
</table>

Values are expressed as the mean (±S.E.M.) of four separate binding experiments performed in duplicate.
Antidiuresis of OPC-51803 in Dogs

The affinity of OPC-51803 for V₂ receptors was about 43 times higher than that for V₁a receptors. Unlabeled AVP displaced [³H]AVP binding to both receptor subtypes with similar Kᵢ values of about 1 nM. DDAVP, which is well known as a selective V₂ agonist, displaced [³H]AVP binding to canine V₂ and V₁a receptors; the affinity for V₂ receptors was 1308 times higher than that for V₁a receptors.

Antidiuretic action of single, oral OPC-51803 dose in water-loaded male dogs

In conscious beagle dogs, a bolus oral administration of water followed by an intravenous infusion of a hypotonic solution led to continuous increases in urine excretion: Excreted urine volume increased to about 20 ml·5 min⁻¹, and urinary osmolality decreased to less than 100 mOsm·kg⁻¹. In the vehicle-treated group, neither urine volume nor urinary osmolality changed throughout the experiment. Orally administered OPC-51803 decreased urine volume and increased urinary osmolality in a dose-dependent manner (Fig. 1). At doses of 0.1 and 0.3 mg·kg⁻¹, significant decreases in urine volume were seen just after 15 min postdose.

Effects of intravenous OPC-51803 infusion on hemodynamics and renal functions in anesthetized dogs

OPC-51803 was intravenously infused at doses of 0.3 and 3 μg·kg⁻¹·min⁻¹. Compared with the vehicle-treated group, a significant decrease in urine volume and an increase in urinary osmolality was seen in the group treated with 3 μg·kg⁻¹·min⁻¹ (Fig. 2). Systemic hemodynamic parameters, such as MAP and HR, remained unchanged (Fig. 3). Statistically significant

Fig. 1. Antidiuretic action of OPC-51803 administered orally to water-loaded conscious dogs. Urine flow (upper) and urinary osmolality (lower) over 5-min periods are shown. Values are expressed as the mean (±S.E.M.) of five dogs. Differences between each OPC-51803-treated group and the vehicle-treated group were statistically analyzed by repeated measures ANOVA. Any significant differences were then analyzed by a two-tailed Dunnett’s multiple comparison test at each time. *P<0.05, **P<0.01.

Fig. 2. Effects of OPC-51803 on urine flow and urinary osmolality in anesthetized dogs. Values are expressed as the mean (±S.E.M.) (n=7). Differences between each OPC-51803-treated group and the vehicle-treated group were statistically analyzed by repeated measures ANOVA. Any significant differences were then analyzed by a two-tailed Dunnett’s multiple comparison test at each time. *P<0.05, **P<0.01.
differences were not seen, either, in GFR or RBF, which is an index of renal functions, between the vehicle-treated group and OPC-51803-treated groups (Fig. 3). The effects of OPC-51803 on urinary parameters are shown in Table 2. At 3 $\mu$g·kg$^{-1}$·min$^{-1}$, OPC-51803 significantly decreased urinary urea nitrogen excretion at the first and second periods after drug infusion was initiated. Although there was a tendency for decreased urinary sodium excretion, it remained statistically insignificant. OPC-51803 did not affect hematocrit, plasma osmolality, or plasma concentrations of AVP, sodium, potassium, or urea nitrogen (Table 3).

**Discussion**

We demonstrated that OPC-51803, a nonpeptide, is an AVP V$_2$ agonist in dogs. After using platelets and kidney medulla membranes to evaluate the affinities of OPC-51803 for canine V$_1a$ and V$_2$ receptors (18, 19), the drug showed an affinity for both receptors. The K$_i$
value of OPC-51803 for V<sub>2</sub> receptors was of the same order as those for rat (49.8 ± 8.1 nM) and human (91.9 ± 10.8 nM) V<sub>2</sub> receptors (1, 2). OPC-51803 showed an affinity for canine V<sub>1a</sub> receptors but the affinity was lower than that for V<sub>2</sub> receptors. A peptide AVP V<sub>2</sub>-agonist, DDAVP, also showed a higher affinity for V<sub>2</sub> receptors. These data show that, as with DDAVP, OPC-51803 has a selective affinity for V<sub>2</sub> receptors in dogs.

As for antidiuretic effects, orally administered OPC-51803 dose-dependently decreased urine volume and increased urinary osmolality in water-loaded dogs. Identical results reported show that AVP and its analogs have V<sub>2</sub> agonistic activities (20). From these results, we confirm OPC-51803’s antidiuretic action in dogs (as with rats) via V<sub>2</sub>-receptor stimulation.

Next, we evaluated the influences of OPC-51803 on systemic hemodynamics and renal functions in anesthetized dogs. OPC-51803 was intravenously infused to maintain constant plasma OPC-51803 concentrations. At 3 μg·kg<sup>−1</sup>·min<sup>−1</sup>, which induced significant antidiuresis, OPC-51803 scarcely exerted a response on MAP or HR. Aki et al. (21) reported that after preadministering a V<sub>1</sub>-receptor antagonist, an intrarenal infusion of AVP caused RBF to increase without pressor action in pentobarbital-anesthetized euvoeomobile dogs. Naitoh et al. (22) also showed that an intravenous infusion of DDAVP significantly increased RBF in conscious dogs via V<sub>2</sub>-receptor activation. In the present study, OPC-51803 tended to increase RBF in the first period of drug infusion, but the changes were not statistically significant. In order to clarify renal vasodilation via V<sub>2</sub>-receptor stimulation induced by OPC-51803, more detailed estimation may be needed. OPC-51803 significantly decreased urinary excretion of urea. AVP reportedly activates urea transporters via V<sub>2</sub>-receptors and promotes urea reabsorption in the inner medullary collecting duct in rats (23, 24). In the same way, OPC-51803 may induce urea reabsorption through the activation of urea transporters via V<sub>2</sub>-receptors, thereby decreasing urinary urea nitrogen excretions in dogs.

In conclusion, OPC-51803 has an affinity for V<sub>2</sub> receptors and shows antidiuresis after oral administration, but has little influence on systemic hemodynamics or renal functions in dogs. At present, synthetic AVP and DDAVP are used in patients with central diabetes insipidus and some kinds of nocturnal enuresis, nocturia,
and polyuria (25–30). It has also been reported that orally administered DDAVP decreased urine volume in Brattleboro rats (AVP-deficient), water-loaded dogs, and even patients with central diabetes insipidus, although DDAVP is a peptide compound (20). However, oral DDAVP presents problems such as large inter- and intraindividual variation (31, 32) attributed to low bioavailability (e.g., less than 1% in humans) (33, 34). Finally, OPC-51803 is absorbed well and shows oral effectiveness in vivo. Therefore, OPC-51803 may provide a new treatment for patients with pathophysiological AVP deficiency.

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References


16 Cheng Y, Prusoff WH. Relationship between the inhibition constant (KI) and the concentration of inhibitor which causes 50 per cent inhibition (Io) of an enzymatic reaction. Biochim Biophys Acta. 1973;22:3099–3108.


26 Asplund R, Aberg H. Diurnal variation in the levels of anti-


