Effects of OPC-21268, an Orally Effective Vasopressin V₁ Receptor Antagonist in Humans

Tsutomu Imaizumi, Seiki Harada, Yoshitaka Hirooka, Hiroyuki Masaki, Michiko Momohara, and Akira Takeshita

An orally effective, nonpeptide vasopressin V₁ receptor antagonist, OPC-21268 was produced for possible human use. We investigated the effects of OPC-21268 on the vascular effects of intra-arterially infused arginine vasopressin in human forearm vessels. The brachial artery was cannulated for drug infusions and direct measurement of arterial pressure. Forearm blood flow was measured by a strain gauge plethysmograph, and forearm vascular resistance was calculated. Arginine vasopressin was infused intra-arterially at doses of 0.02, 0.06, 0.09, 0.2, 0.6, and 1.2 ng/kg/min. The lower doses of arginine vasopressin increased, whereas the higher doses of arginine vasopressin decreased forearm vascular resistance (p<0.01). Intra-arterial infusion of phenylephrine at doses of 0.2, 0.4, and 2.4 μg/min increased forearm vascular resistance dose-dependently (p<0.01). OPC-21268 (50 mg for two, 100 mg for six, and 200 mg for two subjects) given orally did not alter resting arterial pressure, forearm vascular resistance, or heart rate. OPC-21268 decreased vasoconstrictor responses to arginine vasopressin at doses of 0.02 (p<0.02) and 0.09 (p<0.05) ng/kg/min and augmented vasodilator responses to arginine vasopressin at a dose of 1.2 ng/kg/min (p<0.01). However, the vasoconstrictor responses to phenylephrine were not altered by OPC-21268. These results demonstrated that OPC-21268 effectively and specifically antagonized the V₁ receptor-mediated vasoconstriction in human forearm resistance vessels. These results suggest that OPC-21268 may be useful therapeutically to antagonize the vasoconstriction caused by arginine vasopressin in some pathological states.

KEY WORDS • arginine vasopressin • receptors, vasopressin • vasopressins • plethysmography • forearm • human studies

Arginine vasopressin (AVP) causes vasoconstriction via V₁ receptor-mediated mechanisms. AVP is elevated in malignant hypertension and hypertension associated with chronic renal failure. An intravenous specific V₁ receptor antagonist, d(CH₂)Tyr⁵(Me)-AVP (Manning compound), decreases blood pressure in these conditions. AVP is also elevated in congestive heart failure. The Manning compound decreased peripheral resistance and increased cardiac output and dP/dt in some patients with congestive heart failure and a plasma level of AVP more than 4 pg/ml. Thus a V₁ receptor antagonist may be useful for treatment in some types of hypertension and congestive heart failure.

Although many vasopressin antagonists have been developed, these antagonists are all peptide analogues and therefore do not have enough oral bioavailability. Recently, an orally effective, nonpeptide vasopressin V₁ receptor antagonist has been produced for possible human use. The effects of OPC-21268 on AVP-induced hypertension were examined in conscious rats. Vasoconstriction induced by intravenous AVP (30 mg/kg) was inhibited in dose-dependent and time-dependent manners by oral administration of OPC-21268. The effects of OPC-21268 lasted for more than 8 hours at 30 mg/kg, and the 50% inhibition dose (ID₅₀) for AVP-induced vasoconstriction was estimated to be 2 mg/kg. However, there are no available human data on OPC-21268. In the present study, we examined effects of OPC-21268 on vascular responses to intra-arterially infused AVP and measured forearm blood flow in healthy humans.

Methods

General Procedures

Subjects were all young healthy male volunteers (20–23 years old). The protocol was explained, and informed written consent was obtained from each subject. The study was approved by the ethical committee for human study. The subjects underwent routine physical examination, blood tests, chest roentgenography, and electrocardiography, which were all within normal limits. The study was done with subjects in a supine position and in a postabsorptive state in an air-conditioned room with room temperature of about 23°C. Under local anesthesia with 2% procaine, the left brachial artery was cannulated with a 20-gauge intravascular over-the-needle PTFE catheter (Quick-Cath, Travenol Laboratories, Inc., Baxter Healthcare Corporation, Deerfield, Ill.) for drug infusion. The catheter...
was also connected by a three-way stopcock to a pressure transducer (Viggo-Spectramed, Oxnard, Calif.) for direct measurement of arterial pressure. The arterial line was kept open by infusing heparinized saline (0.1 ml/min) when no drug was being administered. A vein in the antecubital region of the same arm was cannulated with the same cannula as that used to obtain blood samples from the vein for measuring plasma levels of AVP and concentration of OPC-21268. Heart rate was obtained by counting the pulse rate for a few minutes on arterial pressure recordings.

Measurements of Forearm Blood Flow
Forearm blood flow was measured by using a mercury-in-Silastic strain gauge plethysmograph with a venous occlusion technique. The strain gauge was placed approximately 5 cm below the antecubital crease. Forearm blood flow (milliliters per minute per 100 milliliters forearm) was calculated from the rate of increase in forearm volume while venous return from the forearm was prevented by inflating the cuff on the upper arm. The pressure in the venous occlusion or congesting cuff on the upper arm was 40 mm Hg. Circulation to the hand was arrested by inflating a cuff around the wrist. The wrist cuff was inflated before the determination of forearm blood flow and continuously throughout the measurements. Forearm vascular resistance was calculated by dividing the mean arterial pressure (diastolic pressure plus one-third of the pulse pressure in millimeters of mercury) by the forearm blood flow. These values are expressed as units throughout this report. An average of four flow measurements made at 15-second intervals, which were calculated by two authors independently, was used for later analysis.

Forearm Vascular Responses to Drugs
After placement of the cannulas and a strain gauge plethysmograph, at least 15 minutes were allowed for subjects to become accustomed to the study condition before beginning the experiments.

We examined responses to intra-arterial infusion of AVP (n = 10) and phenylephrine (n = 10) at graded doses before and after OPC-21268. First, we examined forearm responses to intra-arterial infusions of AVP (0.02, 0.06, 0.09, 0.2, 0.6, and 1.2 ng/kg/min) or phenylephrine (0.2, 0.8, and 2.4 μg/min) for 2 minutes at each dose. Infusions of these drugs were alternated, and infusion of the second drug was begun at least 15 minutes after termination of the first drug when the forearm blood flow had returned to the baseline value. OPC-21268 (50 mg for two, 100 mg for six, and 200 mg for two subjects) was given orally and 30 minutes later infusions of AVP or phenylephrine were begun. Infusions of AVP and phenylephrine were alternated. All infusions of drugs were finished within 1½ hours after administration of OPC-21268. Forearm blood flow was recorded continuously during infusion of drugs. The volume and concentration of arginine and phenylephrine infusion were adjusted so that the infusion volume did not exceed 0.6 ml/min. We had confirmed that this volume of infusion by itself did not alter forearm blood flow. The last 1-minute of measurements of forearm blood flow during infusion of each dose of drugs was used for later analysis.

Measurements of Plasma Arginine Vasopressin and Concentration of OPC-21268
Five milliliters blood was drawn for measurements of AVP during the control period and during infusions of each dose of AVP except the dose of 0.2 ng/kg/min. Blood was sampled into a tube containing EDTA-2K (1 mg/ml). Five milliliters blood was drawn for measurements of the concentration of OPC-21268 during the control period, 30 minutes after oral administration of OPC-21268, and at the end of AVP and phenylephrine infusion. Blood samples were centrifuged immediately and stored in a freezer at −20°C. Drug concentration and plasma AVP were measured by radioimmunoassay at Otsuka Pharmaceutical Co., Tokushima, Japan. Plasma AVP was measured with the method described by LaRochelle et al who used small columns packed with octadecasyl-silica for radioimmunoassay.

Preparation of Drugs
Synthetic AVP (20 pressor units/ml) (Pitressin, Parke-Davis, Inc., Morris Plains, N.J.) was dissolved in physiological saline (10 ml) immediately before use. One milligram phenylephrine (Neo-synesin Kowa, Kouwa, Nagoya, Japan) was dissolved in physiological saline.

Statistical Analysis
The resting hemodynamic values before infusion of AVP and phenylephrine, before and after OPC-21268, were compared by one-way of analysis of variance (ANOVA). The percent changes in forearm vascular resistance induced by AVP and phenylephrine before and after OPC-21268 were compared by two-way ANOVA. When they were significantly different, values at the same dose were compared by paired t test. Since the number of subjects who took 50 mg (n = 2) and 200 mg (n = 2) OPC-21268 was small, all data (n = 10) were analyzed together. All values were expressed as mean±SEM, and p<0.05 was considered to be statistically significant.

Results
Responses to Intra-arterial Infusion of Arginine Vasopressin
Direct intra-arterial infusion of AVP at doses of 0.02, 0.06, 0.09, 0.6, and 1.2 ng/kg/min increased the plasma AVP from 1.8±0.6 (n = 10) to 4.9±1.6 (n = 6), 38.1±8.4 (n = 6), 50.7±10.6 (n = 8), 290.8±99.4 (n = 6), and 489.3±150.6 (n = 8) pg/ml, respectively, in the venous effluents of the ipsilateral arm of AVP infusion. Intra-arterial infusion of AVP caused biphasic changes in forearm vascular resistance (Figure 1). AVP increased forearm vascular resistance at the lower doses and decreased forearm vascular resistance at the higher doses (p<0.01 by one-way ANOVA) (Figure 1). Arterial pressure and heart rate were not altered during infusion of AVP (the data are not shown).

Responses to Intra-arterial Infusion of Phenylephrine
Direct intra-arterial infusions of phenylephrine (n = 10) at doses of 0.2, 0.8, and 2.4 μg/min increased forearm vascular resistance dose-dependently (p<0.01 by one-way ANOVA) (Figure 2). Arterial pressure and
Effects of OPC-21268

The plasma concentration of OPC-21268 of each subject is shown in Figure 3. Since the number of subjects administered 50 and 200 mg was small, statistical analysis was not performed to examine dose-dependent effects. The mean plasma concentration of OPC-21268 before starting drug infusion at the end of the first drug infusion and at the end of the second drug infusion was 2.3±0.3, 3.7±0.7, and 3.6±0.9 μg/min, respectively (not significant by one-way ANOVA). Thus, the plasma concentration of OPC-21268 was stable during the study except in one subject (M.T.) who was administered 200 mg. In this particular subject, the plasma concentration increased with time and reached a very high level.

OPC-21268 altered the forearm vascular responses to AVP (p<0.0046 by two-way ANOVA). OPC-21268 (n=10) decreased vasoconstrictor responses to intra-arterial AVP at doses of 0.2 (p<0.02) and 0.09 (p<0.05) ng/kg/min and augmented vasodilator responses at 1.2 ng/kg/min (p<0.01) (Figure 1). We analyzed separately the results of administering 100 mg OPC-21268 (n=6), and the results were similar to those of the pooled data (n=10). The vasoconstrictor responses to AVP at 0.02 ng/kg/min were smaller (p<0.02) and the vasodilating responses to AVP at 1.2 ng/kg/min were greater (p<0.05) after administration of 100 mg OPC-21268 (n=6). The vasoconstrictor responses to phenylephrine (n=10) were not altered with OPC-21268 (Figure 2).

Adverse Effects

Subjects did not report any adverse effects after taking OPC-21268. There were no detectable changes in blood cell count, urinalysis, or biochemical values 24 hours later.

Discussion

Our results demonstrated that oral OPC-21268 effectively antagonized AVP-induced forearm vasoconstriction but did not affect phenylephrine-induced forearm vasodilation. The vasodilator effects of AVP were augmented after OPC-21268. Our results suggest that

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**Table 1. Resting Hemodynamic Values**

<table>
<thead>
<tr>
<th></th>
<th>Before OPC-21268</th>
<th>After OPC-21268</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVP control</td>
<td>PE control</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>94±3</td>
<td>94±3</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>60±2</td>
<td>59±2</td>
</tr>
<tr>
<td>FBF (ml/min/100 ml)</td>
<td>4.5±0.4</td>
<td>5.0±0.4</td>
</tr>
<tr>
<td>FVR (units)</td>
<td>22.5±2.2</td>
<td>19.9±1.6</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n=10. AVP, arginine vasopressin; PE, phenylephrine; MAP, mean arterial pressure; HR, heart rate; bpm, beats per minute; FBF, forearm blood flow; FVR, forearm vascular resistance; ANOVA, analysis of variance; NS, not significant.
Biphasic Vascular Responses to Intra-arterial Arginine Vasopressin

AVP is a potent vasoconstrictor in vitro. However, AVP does not produce the expected rise in blood pressure when given intravenously to intact animals or to humans. Several mechanisms are considered to account for the discrepancy of the effects of AVP between in vitro and in vivo studies. These include withdrawal of the sympathetic tone due to augmented baroreceptor reflexes and/or other mechanisms; negative inotropic and chronotropic actions; and direct vasodilation. In humans, it has been shown that intravenous AVP markedly decreases sympathetic nerve activity to the skeletal muscle with minor changes in arterial pressure. Recently we and Hirsch et al have shown in humans that direct intra-arterial infusion of AVP causes forearm vasodilation. In the present study, we again demonstrated the direct vasodilating effect of AVP. Although the present study did not aim to elucidate the mechanisms of AVP-induced vasodilation, discussion on this point may be relevant.

Hirsch et al examined vasodilating effects of intra-arterial AVP after a specific intravenous V1 receptor antagonist and found that the vasodilating effects of AVP were potentiated. In addition, they examined effects of intra-arterially infused V1 agonist, 1-deamino, [D-Arg8]vasopressin on forearm blood flow. The V1 agonist increased forearm blood flow. From those results, they suggested that the vasodilating effect of AVP is mediated by the V1 receptor. Our results are compatible with those of Hirsch et al because the vasodilating effect of intra-arterial AVP was potentiated after treatment with OPC-21268, a specific V1 receptor antagonist. To clarify this point in a decisive way, we will need to examine whether a V1 receptor antagonist blocks AVP-induced vasodilation.

Effects of OPC-21268 on Arginine Vasopressin–Induced Vasoconstriction

AVP causes V1 receptor–mediated vasoconstriction. Effects of intravenous OPC-21268 on AVP-induced vasoconstriction were examined in pithed rats. OPC-21268 at doses of 1, 3, and 10 mg/kg did not alter pressor responses to angiotensin II or norepinephrine, but OPC-21268 produced dose-dependent inhibition of pressor responses to AVP (30 milliunits/kg). Furthermore, OPC-21268 at doses of 0.1, 0.3, and 1.0 mg/kg i.v. produced rightward parallel shifts of the dose–response curves for AVP in a dose-dependent manner. These results suggested that OPC-21268 competitively and specifically antagonizes pressor responses to AVP in vivo. OPC-21268 was effective after oral administration as well. After oral administration of OPC-21268 to conscious rats, vasoconstriction induced by exogenous AVP was inhibited in a dose- and time-dependent manner. The effect of oral OPC-21268 at 30 mg/kg lasted more than 8 hours. The ID50 for AVP-induced vasoconstriction was estimated to be 2 mg/kg. Thus, studies in animals suggest that OPC-21268 effectively inhibits AVP-induced vasoconstriction. However, no human data have been available. In the present study, the lower doses of intra-arterial AVP caused vasoconstriction of forearm vessels, which was effectively attenuated by oral OPC-21268. In contrast, OPC-21268 did not affect vasoconstriction induced by intra-arterial phenylephrine. Thus, our results suggest that oral OPC-21268 effectively and specifically antagonizes V1 receptor–mediated vasoconstriction in humans.

Two hundred milligrams OPC-21268 was given to two subjects, 100 mg to six subjects, and 50 mg to two subjects. Since the total number of subjects was small, we did not examine dose-dependent effects of OPC-21268 on vascular responses to AVP. However, when the effects of 100 mg OPC-21268 (n=6) on AVP-induced vasoconstriction and vasodilation were separately analyzed, results were similar to those of the pooled data. Thus, 100 mg OPC-21268 was effective in antagonizing the forearm vasoconstrictor effects of AVP in humans. It is not clear from our study whether 50 mg OPC-21268 was effective in antagonizing AVP-induced vasoconstriction. In conscious rats, OPC-21268 at 10 mg/kg inhibited AVP-induced vasoconstriction by 80%, and the plasma level of OPC-21268 at 10 mg/kg was 1.22 µg/ml (unpublished data from Otsuka Pharmaceutical Co.). In the present study, the plasma concentration of OPC-21268 at 50 mg/kg was 1.1 and 2.0 µg/kg 1 hour after administration, which suggests that 50 mg OPC-21268 may also be effective in humans.

The effect of OPC-21268 on the vasoconstrictor response to AVP at 0.06 ng/kg/min was small. There are some possibilities to account for this. First, the number of the subjects was relatively small; the effect of OPC-21268 may become statistically significant with more subjects. Second, intra-arterial AVP causes biphasic changes in forearm vascular resistance; however, the point at which the vasoconstrictive effects of AVP shifted to the vasodilative effects varied among subjects. Furthermore, in some subjects, the peak vasoconstriction occurred at 0.09 ng/kg/min before OPC-21268 and...
the peak vasoconstriction occurred at 0.06 ng/kg/min after OPC-21268. Thus, the point of peak vasoconstriction shifted to after OPC-21268 administration in some subjects. We speculate that these variations may have caused the minor effect of OPC-21268 on the vasoconstrictor response to AVP at 0.06 ng/kg/min.

OPC-21268 did not alter the baseline forearm hemodynamics in our subjects. Hirsch et al. reported similar findings that a specific V1 receptor antagonist did not alter baseline forearm hemodynamics. Thus, our and their results suggested a lack of tonic vasoconstrictor influence of AVP in healthy subjects.

Clinical Implications

Some patients with malignant hypertension and hypertension associated with chronic renal failure and patients with congestive heart failure have high levels of plasma AVP. The plasma levels of AVP in these patients are mostly in the range below 10 pg/ml and rarely exceed 40 pg/ml. Since OPC-21268 was effective in antagonizing the vasoconstricting effects of AVP at a plasma concentration of 50 pg/ml, one might speculate that OPC-21268 would improve hemodynamics in such pathological conditions by antagonizing effects of AVP. Furthermore, after a single oral dose OPC-21268 was well tolerated, and the effect lasted for at least 1½ hours.

References

Effects of OPC-21268, an orally effective vasopressin V1 receptor antagonist in humans.
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