Potent Aquaretic Agent

A Novel Nonpeptide Selective Vasopressin 2 Antagonist (OPC-31260) in Men


*Department of Internal Medicine (1), Daisan Hospital, The Jikei University School of Medicine, Tokyo 201; *College of Bio-Medical Technology, Osaka University, Toyonaka, Osaka 560; *Tokyo Pharmacological Research Center, Tokyo 171; and *Second Tokushima Institute of New Drug Research, Otsuka Pharmaceutical Co., Tokushima 771-01, Japan

Abstract

Solute-free water diuretics (aquaretics) by antagonizing hydromotic vasopressin receptors (V2) may be useful in treating water-retaining diseases. The effects of intravenous administration of a newly developed nonpeptide, selective V2 antagonist, OPC-31260, at doses ranging from 0.017 to 1.0 mg/kg to groups of healthy, normally hydrated men were compared with those of 0.33 mg/kg furosemide and placebo. OPC-31260 increased the hypotonic urine volume dose dependently for the first 4 h, while furosemide induced sodium diuresis for 2 h. The absolute increase in the cumulative response in the urine to the highest doses of OPC-31260 was not significantly different from that to furosemide. The higher doses of OPC-31260 rapidly lowered urine osmolality for 2 h, particularly between minutes 15 and 45 (e.g., 1.0 mg/kg dose: 63±2 mOsm/kg in urine collected between minutes 30 and 45). In a marked hypotonic diuresis, mean free water clearance of the 4-h urine increased dose proportionally into the positive range, reaching 1.80±0.21 ml/min at 1.0 mg/kg. Whereas furosemide induced marked Na and K diuresis, OPC-31260 increased urinary Na excretion only slightly. At 4 h, 0.75 and 1.0 mg/kg of OPC-31260 almost doubled the plasma arginine vasopressin; and the higher doses increased plasma osmolality and plasma Na slightly, but did not alter plasma K, blood pressure, or heart rate. OPC-31260 thus safely induced a potent aquaretic effect in men. (J. Clin. Invest. 1993, 92:2653–2659.) Key words: vasopressin antagonist • aquaretic effect • urine osmolality • hyponatremia • arginine vasopressin

Introduction

Solute-free water diuretics are required for treating various hydrotropic hyponatremic disorders. One such disease manifesting hyponatremia is the syndrome of inappropriate antidiuretic hormone secretion (SIADH)1 accompanied with tursorous secretion of arginine vasopressin (AVP) and pulmonary or central nervous disorders. Congestive heart failure and liver cirrhosis also manifest hypervolemic, hypotonic hyponatremia, which has been demonstrated to be responsible for the increased AVP effect on V2 AVP renal tubular receptors (1–3). In such disease states, however, failure to suppress the action of AVP in the kidney and the limitation imposed on water excretion have contributed to the difficulty of treating these conditions. There has been an obvious need to develop a potent selective V2 receptor antagonist that can be safely administered over the long term in a clinical setting.

OPC-31260 ((±)-5-dimethylamino-1-(4-[2-methylbenzoylamino]benzoyl)-2,3,4,5-tetrahydro-1H-benzazepin hydrochloride) is a novel nonpeptide, selective V2 receptor antagonist that is now under clinical development in Japan as a potential therapeutic agent that induces solute-free water diuresis (aquaresis). Although several peptide AVP antagonists are currently available, their aquaretic activities have been proven to be limited or absent, because of their partial agonist activities (4–6) and species-difference properties (6–8) in man. OPC-31260 has been demonstrated, however, to possess no partial agonist activity and to exert an aquaretic effect in several animal species (9). Further, in in vitro studies, OPC-31260 is ~100 times more selective for V2 receptors than for V1 receptors (9).

In this study we investigated the aquaretic responses to a series of doses of this new agent and evaluated its safety in men, in comparison with that of the most common loop diuretic, furosemide, after a single administration. Our results suggest that this nonpeptide agent is a potent aquaretic agent in men and a promising pharmacological tool for the treatment of hyponatremic, water-retaining diseases.

Methods

A total of 32 healthy male volunteers, 20–36 yr of age (mean±SD, 23.8±2.8 yr old) and weighing between 55.0 and 76.5 kg (mean±SD, 62.7±6.3 kg), participated in the study after giving informed consent. The study protocol was approved by the Institutional Ethics Committee. Each subject was judged to be healthy on the basis of a complete physical examination, a 12-lead electrocardiogram (ECG), hemogram, blood chemistry profile, and urinalysis. All subjects were normotensive and had a normal heart rate (HR). No subject was exposed to any other medication throughout the study. Each of them entered the Research Ward of Tokyo Clinical Research Center at least 24 h before the study began, where humidity (60%) and temperature (26°C) were comfortably regulated, and stayed there throughout each stage of the study period: days 1–3 in each stage.

During their stay they were advised to adhere strictly to a fixed diet (~2,300 calories, 200 mEq sodium, 70 mEq potassium, and 1,600 ml water contained in the meals, per d), and to avoid sodium additives. They were also prohibited from taking baths or excessive exercise, cof-
fee, tea, or green tea, or xanthine-containing beverages, which could alter the diuretic effect. We set day 1 as a preliminary observation day. We checked the subjects' water intake outside mealtimes (asking them to take a sodium- and caffeine-free drink, usually barley tea, whenever desired) and their daily urine output throughout the study period. On day 2, the baseline day, after an overnight fast, the subjects emptied their bladders (7:00 a.m.), a venous catheter was inserted into an antecubital vein for the intravenous administration of placebo (9:00 a.m.), and blood samples were taken for plasma osmolality, plasma electrolytes (Na, K, Cl), plasma AVP, plasma renin activity (PRA), and plasma aldosterone (PA) up to 24 h after administration. The recumbent state was maintained for 2 h before dosing and for 4 h after dosing, except when urine was voided, and hemodynamics (blood pressure [BP] and HR) and electrocardiograms were monitored at fixed time intervals during the study period. Timed urine collections were made 2 h before dosing and 15, 30, and 45 min, and 1, 1.5, 2, 4, 6, 8, 12, and 24 h after. Urine was collected for the assessment of urine volume, urinary electrolyte excretion, urinary urea excretion (only for 0.75 and 1.0 mg/kg of OPC-31260), and urinary osmolality. Lunch was served to each subject at 1:00 p.m. (4 h after administration), supper at 6:00 p.m. (9 h after), and a light meal at 9:00 p.m. (12 h after). To avoid intersubject variations of water balance and excessive dehydration due to diuresis, each subject was given 200 ml of barley tea 1 h before dosing and 150 ml of barley tea 2, 4, 6, 8, 9, and 12 h after dosing (total, 1,100 ml), and was then allowed free access to barley tea until 7:00 a.m. the next morning. On day 3, the same procedure was repeated, but this time, an active drug or placebo was given in a single-blind fashion from a contralateral antecubital vein, and blood sampling was conducted in a similar manner to the day 2 procedure.

Furosemide. Eight subjects participated in this study: six subjects received 0.33 mg/kg of furosemide and the remaining two received placebo (0.9% saline). Both groups were intravenously given over 5 min.

OPC-31260. 24 subjects who were randomly divided into 3 groups of 8 subjects each participated alternately in successive steps of the study (a total of six steps). Beginning with the lowest dose, 0.017-, 0.1-, 0.25-, 0.5-, 0.75-, and 1.0-mg/kg doses of OPC-31260 were used. At each step, six subjects received the active compound and two received placebo intravenously over 5 min, each group taking during the study a total of two doses, which were three dose levels apart (e.g., 0.017 and 0.5 mg/kg). A washout period of at least 6 wk separated consecutive dose steps for any one subject. After each step the subjects were checked for urinary response and safety parameters before the study advanced to the next higher dose step.

Analytical measurements. Serum and urinary concentrations of sodium and potassium were measured with an autoanalyzer (ION-150AC; Jokoh Co., Tokyo, Japan) equipped with ion-specific electrodes. Creatinine levels in the serum and urine were assayed by an automated alkaline picrate method using an autoanalyzer (736-10; Hitachi Co., Hitachi, Japan). Serum and urinary osmolalities were measured with a freezing point depression osmometer (3D2; Advanced Instruments, Needham Heights, MA).

Blood samples (5 ml) for determining plasma AVP were drawn into ice-chilled tubes containing Na2-ethylenediamine-tetra-acetic acid (EDTA). Plasma AVP was determined by RIA using an AVP-RIA kit (Mitsubishi Petrochemical Co. Ltd., Tokyo, Japan). The intraassay reproducibility was examined at four plasma vasopressin levels over the range of 1–11 pg/ml and interassay reproducibility, at three plasma vasopressin levels over the range of 1–4 pg/ml. Coefficients of intra assay and interassay variation were 2.5–9.3% (n = 5) and 3.6–7.8% (n = 7), respectively. The measurable plasma concentration ranged from 0.2 to 13.0 pg/ml. All samples were analyzed in duplicate.

Blood samples for determining PRA and PA were collected in prechilled test tubes containing EDTA. PRA and PA were measured by RIA using commercial kits (PRA: Gamma Coat Renin kit; Baxter Corp., MN; PA: SPAC-S Aldosterone kit; Daiichi Radioisotope Laboratory, Tokyo, Japan). Intraassay coefficients of variation were 4.8–7.5% for PRA and 3.8–9.8% for PA.

Data analysis. Free water clearance (CwH2O) in 4- and 24-h urine after dosing was calculated using the following equation:

\[ C_{wH2O} = C_{osmol} - C_{urine} \]

where urine flow and C_{osmol} (osmolar clearance) from 0–4 or 0–24 h are expressed in milliliters/minute. C_{osmol} was calculated from the following equation:

\[ C_{osmol} = \frac{U_{osmol} \times \text{urine flow}}{P_{osmol}} \]

where U_{osmol} = urine osmolality, and P_{osmol} = plasma osmolality.

![Figure 1. Cumulative urine volume-time relationships after administration of one of six doses of OPC-31260 (○, 0.017; ●, 0.1; ▲, 0.25; ◇, 0.5; ●, 0.75; ○, 1.0 mg/kg), of a dose of furosemide (0.33 mg/kg, ○), or of placebo (□ and dotted lines) on day 3. Data indicate mean±SEM for 6 active drug subjects (in each dose) or for 14 placebo subjects. Statistical comparisons with placebo used ANOVA followed by Dunnett's test for individual comparisons. *P < 0.05; **P < 0.01 compared with the matched placebo value.](image-url)
and 3) and 24 h and 10 d after it. All side effects or adverse reactions were noted on the respective case report forms, with a brief description specifying their nature, severity, date, time of occurrence, duration, and the investigator’s opinion of whether the reaction was attributable to the test compound or incidental.

**Results**

**Urine volume.** Fig. 1 shows the relation of cumulative urine volume (CUV) to time in response to intravenous administration of furosemide (0.33 mg/kg), six doses of OPC-31260 (0.017–1.0 mg/kg), or placebo on day 3. Furosemide increased the urine output markedly for 2 h, while OPC-31260 increased the urine volume dose proportionally and more gradually for 4 h. The CUV after furosemide was significantly greater for the first 2 h than those after six doses of OPC-31260. The 0–4 h CUVs after the two highest doses (0.75 and 1.0 mg/kg) of OPC-31260, however, did not differ from that of furosemide. Individual comparison shows that the CUV-time relationship of the smallest OPC-31260 dose (0.017 mg/kg) did not differ from that of placebo, and that the CUVs at 24 h with furosemide and with all six doses of OPC-31260 were not significantly different from that with placebo.

The absolute increases in CUV during hours 0–4 (ΔCUV<sub>0–4</sub>) and hours 0–24 (ΔCUV<sub>0–24</sub>) (day 3 minus day 2) are shown in Fig. 2. The mean value of ΔCUV<sub>0–4</sub> after OPC-31260 administration clearly increased dose proportionally, and the mean ΔCUV<sub>0–4</sub> values at 0.75 and 1.0 mg/kg were not significantly different from that with furosemide. This ΔCUV disappeared when the value was taken over 24 h, and ΔCUV<sub>0–24</sub> values with furosemide and with six doses of OPC-31260 were not significantly different from that with placebo.

**Urine osmolality and free water clearance.** Fig. 3 shows the time courses of urine osmolality after the single doses and clearly demonstrates that urine osmolality dropped rapidly and almost dose dependently until 2 h after intravenous administration of the five doses of OPC-31260 from 0.1 to 1.0 mg/kg. The maximum drops in osmolality were seen in the 15–45-min
urine. The highest dose of this drug (1.0 mg/kg) decreased urine osmolality to 63±2 mOsm/kg in the 30–45-min urine, and this low level lasted for 2 h. Furosemide also decreased urine osmolality for 1 h, but its maximum reduction was only to 255±5 mOsm/kg in the 0–15-min urine. A significant reduction compared with placebo was obtained for 2 h after each of the four highest doses of OPC-31260 (Fig. 3). The urine osmolality decreases after the higher doses of OPC-31260 from 0 to 4 h were significantly greater than those of furosemide (*P < 0.05). The 0.017-mg/kg dose of OPC-31260 did not show any urine osmolality-lowering effect.

Table I summarizes the mean free water clearance (CH2O) in the 4-h and 24-h urine, and clearly shows a major increase of CH2O (0–4 h) in a dose-proportional manner in the positive range (Table I). The mean (±SEM) of CH2O (0–4 h) after administration of 1.0 mg/kg of OPC-31260 was 1.80±0.21 ml/min. The mean CH2O values (0–4 h) of three doses (0.5–1.0 mg/kg) were significantly higher than that of placebo (−0.3±0.13 ml/min) and furosemide (−0.53±0.07 ml/min). However, the mean CH2O (0–24 h) turned negative at all doses of OPC-31260, ranging from −0.29±0.07 to −0.46±0.05 ml/min, values that did not differ significantly from those with placebo (−0.44±0.04 ml/min) or furosemide (−0.55±0.02 ml/min).

Urine electrolys (Na, K, Cl) and urea excretions. Furosemide increased urinary sodium and chloride excretion during the first 4 h (UNa and UC10) to almost three times the level with placebo: 140±8.6 vs. 48.6±4.2 mEq in UNa and 154.2±9.0 vs. 50.7±3.7 mEq in UC10, respectively (*P < 0.01) (Table I). OPC-31260 increased UNa (0–4 h) proportionally, but only to a small degree. UNa (0.75, 1.0 mg/kg) was significantly higher after OPC-31260 than after placebo: 72.1±5.6 and 70.4±4.5 mEq for the two OPC-31260 doses, respectively (Table I). The mean (±SEM) urinary sodium and chloride excretions over 24 h (UNa and UC10) showed almost the same values at all six doses of OPC-31260, furosemide, and placebo, ranging from 159.3±11.8 to 185.±14.8 mEq in UNa and 173.5±7.2 to 208.3±9.2 mEq in UC10, respectively.

During the first 4 h, furosemide administration caused almost twice the urinary potassium excretion (UKa) that was seen after placebo dosing (25.8±2.7 vs. 13.8±1.4 mEq; *P < 0.01), while OPC-31260 did not alter UKa. There was no significant difference in urinary potassium excretion over 24 h (UKa) between the six doses of OPC-31260 and placebo (Table I).

Urea excretion in the 4-h urine did not differ significantly at the highest doses (0.75, 1.0 mg/kg) of OPC-31260 from that with placebo: 59.4±4.8, 48.9±6.6 vs. 47.8±4.0 mmol, respectively.

Hormonal, hemodynamic, and fluid balance. Fig. 4 shows the change in mean plasma AVP level with time after a single dosing. The two highest doses (0.75 and 1.0 mg/kg) of OPC-31260 elevated the plasma AVP level significantly (*P < 0.05), with the maximum at 4 h after dosing of 1.2±0.2–3.1±0.5 and 1.9±0.5–3.1±0.6 pg/mL, respectively. At 24 h, the plasma levels of these doses returned to the baseline values. Table II shows PRA and PA alterations before and 4 h after the administration of the three highest doses of OPC-31260 and of furosemide. Furosemide administration elevated PRA markedly (*P < 0.01) from 2.7±0.6 to 7.6±1.0 ng/mL per h in 4 h. 4 h after administration of these three doses of OPC-31260, PRA showed a nonsignificant tendency to increase from the respec-
tive baseline values before administration. There were no significant changes in PA between the predosing and 4-h values.

Plasma osmolality increased slightly but not significantly 4 h after the intravenous administration of OPC-31260: 0.5 mg/kg, 0.7±0.8%; 0.75 mg/kg, 0.7±0.3%; 1.0 mg/kg, 0.7±0.6% (Table II). However, determination of plasma osmolality between 0 and 4 h showed that the time of the maximum value \( t_{max} \) was < 4 h at each of these doses \( (P < 0.05) \): at 0.5 mg/kg, 285.2±1.2 mOsm/kg at \( t_{max} \) of 1.7±0.5 h showing a 2.0% increase in plasma osmolality; at 0.75 mg/kg, 283.5±1.2 mOsm/kg, 1.8±0.5 h \( t_{max} \), a 1.6% increase; and at 1.0 mg/kg, 283.7±0.6 mOsm/kg, 2.7±0.4 h \( t_{max} \), a 1.7% increase.

Plasma sodium concentration \( (P_{na}) \) increased significantly between 0 and 4 h after the highest doses of OPC-31260 (0.75, 1.0 mg/kg) but, as shown in Table II, there were no significant differences in the plasma potassium level after OPC-31260. The hemodynamics (mean BP and HR) did not change at all after the administration of OPC-31260 or furosemide.

We measured creatinine clearance \( (C_{cr}) \) on days 2 and 3 to verify that there was no alteration of the glomerular filtration rate. There was no alteration in \( C_{cr} \) between these 2 d, all values being within the normal range, whether furosemide or any dose of OPC-31260 was given.

**Safety assessment.** After OPC-31260 administration at the dose of 1.0 mg/kg very slight fatigue from 1 to 9 h after dosing was reported by one subject receiving OPC-31260. No other clinically undesirable signs or symptoms attributable to the administration of OPC-31260 were recognized and no relevant clinical changes in laboratory safety parameters were observed in any subject throughout the study period.

**Discussion**

Successful and safe blockade of V2 renal AVP receptors could offer a promising treatment for various diseases characterized by excessive renal reabsorption of free water due to increased AVP release and/or action, such as SIADH and possibly congestive heart failure or liver cirrhosis. Many efforts have therefore been concentrated on finding a potent and safe aquaretic as a selective V2 receptor antagonist. Investigators have reported many peptide analogues that are V1/V2 receptor antagonists, and recently, Manning et al. (10) have demonstrated that a ring structure in the AVP molecule is not required for antagonizing V1/V2 receptors. Nonetheless, many investigators have modified the structure (11–14), but have as yet found no nonpeptide V1/V2 receptor antagonist. Our previous reports (15,16), however, described a nonpeptide, orally active V1 receptor antagonist, OPC-21268, and then OPC-31260, a nonpeptide V2 receptor antagonist, also has been developed. In vitro animal experiments using \(^{[3]H}AVP\) have demonstrated that OPC-31260 is a selective V2 receptor antagonist, and this agent has been shown to behave as an aquaretic agent in rats (9).

OPC-31260 exerted a powerful aquaretic effect in normally hydrated healthy men. The diuretic effect of this drug (0.75, 1.0 mg/kg) (mainly aquaresis) is almost equipotent to that of furosemide (0.33 mg/kg or 20 mg/60 kg, which is generally accepted as the intravenous adult starting dose for various edematous conditions). Moreover, the urine osmolality—lowering effect of OPC-31260 (0.1–1.0 mg/kg) was maintained for 2 h, and the free water clearance \( (C_{H_2O}) \) increased positively in a dose-proportional manner. A 1.0-mg/kg dose of OPC-31260 increased \( C_{H_2O} \) (0–4 h) to 1.8±0.21 ml/min (mean±SEM), which exhibited a powerful hypotonic diuretic effect in men.

Recently, the V2 receptor antagonist SK&F 101926, a peptide analogue, which shows aquaretic activity in several animal models, failed to induce aquaresis in men, and indeed has been demonstrated to be a potent antidiuretic in human subjects, probably because of its agonistic activity (6). This study has shown that species differences and agonistic activity limit its ability to generate an aquaretic effect by V2 receptor blockade in vivo. As was demonstrated in several animal models in vivo (9), OPC-31260 had an aquaretic effect, and this study shows a similar effect in men, suggesting strongly that the effect of the drug is hardly affected by species differences. In addition, it is unlikely that OPC-31260 has any masked agonistic activity, at least within the present dose range (0.017–1.0 mg/kg). Since Albright-Winslow et al. (17) have reported that cyclooxygenase inhibition unmasked the full antidiuretic agonist activity using SK&F 101926, future studies must verify the aquaretic efficacy of OPC-31260 on treatment with some cyclooxygenase inhibitor. On the other hand, Hofbauer et al. (5) have demon-
stated that chronic administration of a V1/V2 antagonist did not accomplish sustained aquarexia, probably owing to its agonistic activity. Therefore, further studies should also investigate whether such a potent aquaretic effect by OPC-31260 is maintained during long-term administration.

The aquaretic effect is nearly maximal at doses of 0.75–1.0 mg/kg. Since urine osmolality dropped markedly to 63 mOsm/kg after a 1.0-mg/kg OPC-31260 dosing, the tubular diluting capacity may have been exercised to its limit, suggesting that the pharmacological effect of this compound may be limited by this capacity. On the other hand, aquarexia is likely to be limited also by the stimulation of an endogenous system for maintaining systemic homeostasis. One aspect of this system was observed in relation to plasma AVP. The higher doses of OPC-31260 (0.75, 1.0 mg/kg) almost doubled the plasma AVP level at 4 h after administration. Competitive interaction as antagonists for the V2 receptors in the kidney between increased levels of endogenous AVP and OPC-31260 could thus be involved in limiting the aquaretic effect.

What mechanisms are involved in the plasma AVP increases at the higher doses of OPC-31260? There are several possibilities. First, the osmotic receptor cells in the anterior hypothalamus are known to be very sensitive to changes in extracellular fluid osmolality (the osmoregulatory system) (18, 19). Plasma osmolality in this study slightly increased at 4 h (0.5–0.7%). However, it reached higher values before 4 h, 1.6–2.0% increases at tmax of 1.7–2.7 h on administration of 0.5, 0.75, and 1.0 mg/kg of OPC-31260. Robertson et al. (19, 20) have reported that a change in plasma osmolality of only 1% could be expected to change the plasma AVP level by ~1 pg/ml in healthy subjects. Therefore, the increase in plasma AVP in this study could be due to the slight increase in osmolality probably caused by body water loss, that is, by aquarexia by OPC-31260. Nevertheless, the osmolar control of plasma AVP has been known to exhibit considerable individual, genetic, and environmental variation.

Robertson et al. (19, 20) have also pointed out that this osmoregulatory sensitivity is probably rate dependent and requires a few hours to respond. The tmax values of 1.7–2.7 h for plasma osmolality could conceivably have led to the peak levels of plasma AVP at 4 h. However, we cannot explain the AVP increases solely on the basis of osmoregulatory control, and so nonosmotic regulation cannot be ruled out.

Since OPC-31260 is a highly lipophilic agent, it penetrates the blood–brain barrier to stimulate the osmoreceptor cells in the anterior hypothalamus. On the other hand, slight, but not significant, increases in PRA after the administration of higher OPC-31260 doses should indicate increases in the circulating angiotensin II level. Since angiotensin II is one of the putative mediators of AVP release (21, 22), this hormone increase may play a part in incrementing the plasma AVP level. These suggestions are, of course, highly speculative.

Furosemide induced marked increases in urinary electrolyte excretions (Una, Un, and Uc) (0–4 h) because of its ability as a potent loop diuretic. OPC-31260 also elevated Una (0–4 h) significantly, though to a lesser degree. Several reports have demonstrated that AVP enhances sodium reabsorption in the collecting tubules of animal models (23, 24), and so OPC-31260 could inhibit its reabsorption in this portion, resulting in an increase of sodium urinary excretion. Urea-induced osmotic diuresis is unlikely to participate in the diuresis by OPC-31260 because no significant alteration in urinary urea excre-
tion was recognizable when the higher doses of OPC-31260 were given. Thus, it is likely that OPC-31260 acts fundamentally as an aquaretic. Nevertheless, as far as its possible therapeutic use is concerned, more research on the effects of OPC-31260 is required before it can be used for congestive heart failure, liver cirrhosis, and other edematous conditions, particularly when hyponatremia is induced by salt restriction and/or overuse of diuretics.

In conclusion, intravenous OPC-31260 exerts a potent and safe aquaretic effect in men. Its aquaresis and urine osmolality–lowering effect were dose dependent from 0.017 to 1.0 mg/kg and were maintained until 2 h after dosing. The diuresis at the higher doses (0.75, 1.0 mg/kg) was equipotent to that with furosemide (20 mg/60 kg man). This novel drug could be useful for the treatment of SIADH and of other various water-retaining disease conditions.

References


