Effects of the V₂-Receptor Antagonist OPC-41061 and the Loop Diuretic Furosemide Alone and in Combination in Rats

TAKAHIRO HIRANO, YOSHITAKA YAMAMURA, SHIGEKI NAKAMURA, TOSHIYUKI ONOGAWA, and TOYOKI MORI
Second Tokushima Institute of New Drug Research, Otsuka Pharmaceutical Co. Ltd., Kawauchi-cho, Tokushima, Japan
Accepted for publication September 24, 1999

ABSTRACT
This study was conducted to characterize the diuretic effect of OPC-41061, a nonpeptide vasopressin V2-receptor antagonist, and furosemide by administering each alone and in combination in conscious male rats. OPC-41061 at 1 and 10 mg/kg and furosemide at 10 and 100 mg/kg dose-dependently increased urine volume to the same extent. The high dose of OPC-41061 (10 mg/kg) markedly elevated electrolyte-free water clearance (E-CH₂O) to a positive value. In contrast to OPC-41061, furosemide elevated only electrolyte clearance but not E-CH₂O. The differences in diuretic profile reflected the changes in serum sodium and hormone levels. OPC-41061 dose-dependently elevated serum sodium concentration, but furosemide tended to decrease it. The high dose of furosemide (100 mg/kg) significantly elevated serum renin activity and aldosterone concentration, indicating that furosemide activated the renin-angiotensin-aldosterone system (RAA-system). On the other hand, OPC-41061 did not affect these parameters. When OPC-41061 was administered concomitantly with furosemide, OPC-41061 significantly increased urine volume and E-CH₂O, and decreased urinary osmolality compared with furosemide alone. OPC-41061 dose-dependently elevated serum osmolality and sodium concentration even when administered in combination with the high dose of furosemide. These results suggest that OPC-41061 produces aquaresis leading to increased serum sodium without affecting the RAA-system. On the other hand, furosemide produced natriuresis, leading to decreased serum sodium level and activation of the RAA-system. It was also demonstrated that OPC-41061 produced an additive diuretic effect and elevated serum sodium level in the presence of furosemide.

Numerous diuretic agents have been used for the treatment of some edematous states associated with congestive heart failure, liver cirrhosis, and nephrotic syndrome (Morrison, 1997). The conventional diuretics, such as loop diuretics, thiazide diuretics, potassium-sparing diuretics, and carbonic anhydrase inhibitors, produce natriuresis leading to loss of extracellular sodium with water following. However, because of the mechanism of action of these agents, patients taking these conventional diuretics frequently develop hyponatremia and hypokalemia. Furthermore, some patients with heart failure and liver cirrhosis already have dilutional hyponatremia and hypokalemia, suggesting that natriuretics may exacerbate the condition. Thus, solute-free water diuretics (aquaresics) are preferable for the treatment of these diseases.

Recently, we developed nonpeptide arginine vasopressin (AVP) V₂-receptor antagonists: OPC-31260 (Yamamura et al., 1992; Ogawa et al., 1996) and its congener, OPC-41061 (7-chloro-5-hydroxy-1-[2-methyl-4-(2-methylbenzoylamino) benzoyl]-2,3,4,5-tetrahydro-1H-1-benzazepine) (Yamamura et al., 1998; Kondo et al., 1999). These agents exert an aquaresic effect by blocking the V₂ receptors at the renal collecting ducts and thereby inhibiting water reabsorption. It was demonstrated that OPC-41061 inhibited [³H]AVP binding to rat V₂ receptors (Kᵦ, 1.33 ± 0.30 nM) about 250 times more potently than to rat V₁ receptors and produced aquaresis after single and multiple dosing in rats (Yamamura et al., 1998).

So far, the differences between aquaresis and natriuresis have not been investigated in detail with the same experimental procedures. Therefore, we compared the diuretic effects of OPC-41061 and furosemide, the most commonly used loop diuretic, alone and in combination in conscious male Sprague-Dawley rats. In particular, we focused on the effects of these agents on serum parameters, which affect the conditions of the diseases mentioned, such as sodium and AVP levels, and the renin-angiotensin-aldosterone system (RAA-system).

Materials and Methods

Animals. Male Sprague-Dawley rats, aged 7 weeks, were purchased from Charles River Japan, Inc., (Yokohama, Japan) housed in a temperature-, humidity-, and light-controlled room and given free

ABBREVIATIONS: AVP, arginine vasopressin; E-CH₂O, electrolyte-free water clearance; E-Cosm, electrolyte clearance; RAA-system, renin-angiotensin-aldosterone system.
access to food and water. Experimental protocols concerning the use of laboratory animals followed the Guidelines for Animal Experimentation in Otsuka Pharmaceutical Co., Ltd.

**Treatments and Measurements.** The rats, weighing 470 to 530 g (aged 13 weeks), were stratified by body weight and randomized into nine groups. According to the groups listed in Table 1, each agent alone and combination of OPC-41061 and furosemide was administered orally to the rats via a stomach tube. Immediately after administration, the rats were placed individually in metabolic cages (Sugiyama-Gen Medical Instruments Co., Ltd., Tokyo, Japan), and spontaneously voided urine was collected for 4 h. The rats were given neither water nor food during the urine-sampling period. After the volume of collected urine was measured, a portion was centrifuged (hvac CPT7D; Hitachi, Tokyo, Japan) at 1870g for 10 min. The supernatant was used for measurement of urinary parameters. After the urine sampling, the rats were decapitated, and trunk blood was collected into a tube to obtain serum by centrifuging at 1870g for 10 min. Serum and urine concentrations were determined by freezing-point depression with a Fiske osmometer (model 3400; Needham, MA). Serum and urinary sodium concentrations were measured by an ion-electrode method (Synchron CX-3; Beckman Instruments Inc., Fullerton, CA), and creatinine concentration was measured with a COBAS Autoanalyzer (COBAS FARA II; Roche, Basel, Switzerland). Serum AVP and aldosterone concentrations were measured by radioimmunoassay. Serum renin activity was determined by radioimmunoassay with the formation of angiotensin I (expressed in nanograms as an index).

**Calculation.** To clarify the effects of OPC-41061 and furosemide, electrolyte-free water clearance (E-CH_{2}O) and electrolyte clearance (E-Cosm) were calculated. The formulae are

\[
E-CH_{2}O = UV - E-Cosm
\]

\[
E-Cosm = [U_{Na} + U_{K}]UV/S_{Na},
\]

where UV is urine volume, \(U_{Na}\) is urinary sodium concentration, \(U_{K}\) is urinary potassium concentration, and \(S_{Na}\) is serum sodium concentration.

We calculated E-Cosm except the values for serum potassium concentration, because blood sampling was performed by decapitation, leading to hemolysis. The measurement of serum potassium was markedly affected by hemolysis. Moreover, serum potassium concentration is generally low enough to be negligible compared with serum sodium concentration (about 3%).

**Drugs.** OPC-41061 (synthesized at Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) and furosemide (Sigma Chemical Co., St. Louis, MO) were suspended in 1% hydroxypropyl methyl cellulose solution (Shin-etsu Chemical, Niigata, Japan).

**Statistical Analysis.** Values are expressed as means ± S.E. Differences between the groups administered OPC-41061 or furosemide alone and the control group (vehicle) were analyzed by one-way ANOVA, followed by two-tailed Dunnett’s multiple-comparison test. The statistical analysis was performed by use of the Statistical Analysis System (SAS Institute Japan, Tokyo, Japan). Differences were considered significant at \(p < .05\).

**TABLE 1**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 1: comparison tests</strong></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1% HPMC solution (vehicle)</td>
</tr>
<tr>
<td>2</td>
<td>OPC 1 mg/kg</td>
</tr>
<tr>
<td>3</td>
<td>OPC 10 mg/kg</td>
</tr>
<tr>
<td>4</td>
<td>FUR 10 mg/kg</td>
</tr>
<tr>
<td>5</td>
<td>FUR 100 mg/kg</td>
</tr>
<tr>
<td><strong>Experiment 2: combination tests</strong></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>OPC 1 mg/kg + FUR 10 mg/kg</td>
</tr>
<tr>
<td>7</td>
<td>OPC 10 mg/kg + FUR 10 mg/kg</td>
</tr>
<tr>
<td>8</td>
<td>OPC 1 mg/kg + FUR 100 mg/kg</td>
</tr>
<tr>
<td>9</td>
<td>OPC 10 mg/kg + FUR 100 mg/kg</td>
</tr>
</tbody>
</table>

OPC, OPC-41061; FUR, furosemide; 1% HPMC solution, 1% hydroxypropylmethyl cellulose solution. \(n = 6\) group.

**Results**

**Comparison of OPC-41061 and Furosemide.** The doses of OPC-41061 and furosemide were determined where an equal amount of extracellular volume would be reduced. In fact, the low dose (1 mg/kg) and high dose (10 mg/kg) of OPC-41061 increased urine volume to about two and seven times that of the control, respectively (Fig. 1). Furosemide also increased urine volume to about double that at low dose (10 mg/kg) and eight times that at high dose (100 mg/kg).

There were no differences in urine volume between the OPC-41061 and furosemide groups, at both the respective low doses and high doses. Both OPC-41061 and furosemide dose-dependently decreased urine osmolality. OPC-41061 at 10 mg/kg significantly increased urinary sodium excretion, but the increase was half of that in the furosemide group at the high dose. The high doses of both agents significantly increased urinary urea nitrogen excretion (Fig. 1). The high dose of OPC-41061 markedly elevated E-CH_{2}O to a positive value. In contrast, furosemide elevated only E-Cosm but not E-CH_{2}O (Fig. 2). Neither agent affected urinary creatinine excretion (Fig. 1).

OPC-41061 dose-dependently elevated serum sodium concentration at 4 h postdosing. On the other hand, furosemide tended to decrease serum sodium concentration. The high doses of OPC-41061 and furosemide significantly elevated serum osmolality, but the elevation in the OPC-41061 group was double that in the furosemide group. The high doses of OPC-41061 and furosemide elevated serum AVP concentration almost to the same extent. The high dose of furosemide significantly elevated serum renin activity and aldosterone concentration, but OPC-41061 did not affect these parameters. Furosemide significantly increased serum urea nitrogen concentration in a dose-dependent manner, but OPC-41061 did not. The high dose of furosemide significantly increased serum creatinine concentration (Fig. 3).

**Combination of OPC-41061 and Furosemide.** When OPC-41061 (1 and 10 mg/kg) was administered in combination with either the high dose (100 mg/kg) or the low dose (10 mg/kg) of furosemide, OPC-41061 dose-dependently increased urine volume and E-CH_{2}O and decreased urinary osmolality compared with either the high dose or the low dose of furosemide alone. Urinary creatinine excretion was unchanged by the administration of OPC-41061 in combination with furosemide (Fig. 4 and Table 2).

OPC-41061 administered in combination with furosemide dose-dependently increased serum osmolality compared with furosemide alone, and the increase was significant at the high dose of OPC-41061. Whereas furosemide alone tended to decrease serum sodium concentration, OPC-41061 in combination with furosemide significantly increased it. OPC-41061 in combination with furosemide significantly increased serum AVP level only at the high dose of OPC-41061 (Fig. 5). The administration of OPC-41061 in combination with furosemide did not further change serum renin activity and al-
dosterone level (Fig. 5), indicating that there was no additive effect.

**Discussion**

In this study, we focused on the effects of an aquaretic (OPC-41061), a natriuretic (furosemide), and their combination on serum parameters, such as sodium and hormone levels. These parameters are well known to be affected by extracellular volume. Therefore, it is necessary to control the reduction in extracellular volume induced by the tested diuretics at the same level. In our preliminary study, however, OPC-41061 induced water intake more greatly than furosemide, with an identical increase in urine volume. This is because OPC-41061 stimulated the thirst center by elevating serum osmolality. Therefore, the rats were given no water during the urine sampling period (4 h).

Under this condition, OPC-41061 increased urine volume and decreased urinary osmolality in a dose-dependent manner. The diuretic effects of OPC-41061 at 1 and 10 mg/kg were almost equipotent to those of furosemide at 10 and 100 mg/kg, respectively, indicating that OPC-41061 shows almost 10 times more potent diuresis than furosemide in rats. We calculated E-CH₂O instead of the classic CH₂O. Recently, it was reported that E-CH₂O reflected tonicity balance better than classic CH₂O in the treatment of electrolyte-abnormal patients (Shoker, 1994; Mallie et al., 1997). Electrolyte clearance is defined as the osmolar clearance of only effective osmoles (sodium, potassium, and their accompanying anions), except permeable osmoles that do not alter tonicity (e.g., urea). That is, E-CH₂O excludes the spurious effects of the ineffective osmoles on the measurement of free water excretion. The high dose of OPC-41061 markedly increased urea excretion by inhibiting urea reabsorption at the inner medullary collecting duct. Therefore, E-CH₂O is a more suitable index than classic CH₂O in this study. The high dose of OPC-41061 markedly elevated E-CH₂O to a positive value. In contrast, furosemide elevated only E-Cosm but not E-CH₂O. These results suggest that OPC-41061 and furosemide exert an aquaretic and a natriuretic effect, respectively.

The increase in electrolyte excretion induced by the high dose of OPC-41061 was less than that induced by furosemide but was statistically significant. It was previously demonstrated that V₂ antagonists increased sodium excretion in rats (Kinter et al., 1986; Yamamura et al., 1998) but not in dogs (Kinter et al., 1984; Yamashita et al., 1993; Hirano et al., 1994) or monkeys (Kinter et al., 1985). These results are consistent with some reports in vitro that rats and mice possess AVP-sensitive adenylate cyclase activity in the thick
Fig. 3. Effects of oral administration of OPC-41061 or furosemide alone on serum parameters in conscious male rats. Values are expressed as means ± S.E. of six animals in each group. Differences between each OPC-41061 or furosemide group and the control group were analyzed by one-way ANOVA, followed by two-tailed Dunnett’s multiple-comparison test. *P < .05, **P < .01, versus the control.

Fig. 4. Effects of oral administration of OPC-41061 in combination with furosemide on urinary parameters in conscious male rats. Values are expressed as means ± S.E. of six animals in each group. Differences between the groups administered OPC-41061 in combination with furosemide and the groups administered furosemide alone were analyzed by one-way ANOVA, followed by two-tailed Dunnett’s multiple-comparison test. *P < .05, **P < .01, versus administration of furosemide alone.
Thus, because OPC-41061 does not activate the RAA-system, are useful drugs in the treatment of congestive heart failure.

Bailie et al., 1982; Hayashi et al., 1987). Furosemide, of the modulators of renin secretion (Whorton et al., 1980; Abe et al., 1977). It is known that the prostaglandins are one important role in progression of congestive heart failure and liver cirrhosis, because dilutional hyponatremia and hypokalemia frequently develop as congestive heart failure and liver cirrhosis, because dilutional hyponatremia and hypokalemia frequently develop secondary to these diseases. Conventional natriuresis can correct the sodium-retaining states but may exacerbate hyponatremia and hypokalemia. In fact, furosemide tended to decrease serum sodium in this study.

The high dose of furosemide significantly elevated serum renin activity and the aldosterone level. The activation of the RAA-system induced by furosemide is consistent with previous observations in humans (Francis et al., 1985; Anand et al., 1991), and the mechanism has been explained by several investigators as described below. First, furosemide interacts with the Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporters in the thick ascending limb of Henle’s loop. Lorenz et al. (1991) demonstrated that the initiating signal for the macula densa to control of renin secretion is an inverse change in the transport rate via the luminal Na\(^+\)-K\(^+\)-2Cl\(^-\) cotransporters in perfused, superfused preparations of the isolated rabbit juxtaglomerular apparatus. Thus, furosemide is believed to have accelerated renin secretion by interaction with this segment in our study. Second, furosemide stimulates renal production of prostaglandins, such as prostaglandin E\(_2\) (Williamson et al., 1975; Abe et al., 1977). It is known that the prostaglandins are one of the modulators of renin secretion (Whorton et al., 1980; Beierwaltes et al., 1982; Hayashi et al., 1987). Furosemide, therefore, may accelerate renin secretion in part via stimulation of renal prostaglandin production. In fact, Bailie et al. (1976) demonstrated in dogs that indomethacin or meclofenamate prevented the rise in renin release by furosemide. OPC-41061 did not affect serum renin activity or the aldosterone level. It has been established that the RAA-system plays an important role in progression of congestive heart failure and that angiotensin-converting enzyme inhibitors are useful drugs in the treatment of congestive heart failure. Thus, because OPC-41061 does not activate the RAA-system, it may be a desirable diuretic drug for the treatment of edema in congestive heart failure.

The high doses of OPC-41061 and furosemide significantly elevated the serum AVP level to almost the same extent. However, the factors affecting AVP secretion may be partially different. AVP secretion is mainly regulated by serum osmolality and extracellular volume. In this study, the extracellular volume is expected to contribute to the stimulation of AVP secretion induced by both agents to almost the same extent, because the high doses of both agents produced powerful equipotent diuresis under the conditions of restricted access to water. OPC-41061 markedly elevated serum osmolality compared with furosemide. AVP secretion from the anterior hypophalamus is known to be sensitive to changes in extracellular fluid osmolality (Robertson et al., 1973; Berl and Schrier, 1986). Robertson et al. (1973, 1976) reported that a change in plasma osmolality of only 1% could be expected to change the plasma AVP level by 1 pg/ml in humans. Thus, the osmoregulator system seems to contribute to stimulation of AVP secretion, at least in the case of OPC-41061. In the case of furosemide, other mechanisms may be involved in the stimulation of AVP secretion. In this study, furosemide elevated serum renin activity, indicating an increase in the level of circulating angiotensin II. Angiotensin II is one of the humoral mediators of AVP secretion (Padfield and Morton, 1977; Sladek et al., 1982). Thus, the activation of angiotensin II production may also relate to furosemide-induced AVP secretion.

Serum creatinine concentration was increased significantly at a dose of 100 mg/kg of furosemide, suggesting that furosemide decreases glomerular filtration rate. An accurate glomerular filtration rate could not be calculated from serum creatinine concentration and its urinary excretion, because serum creatinine concentration was obtained after hemocoagulation. However, several investigators supported our result that furosemide decreased glomerular filtration rate in rats (Romano et al., 1995; Tenstad and Williamson, 1995). On the other hand, OPC-41061 treatment did not affect serum creatinine concentration. Furosemide significantly increased serum urea nitrogen level in a dose-dependent manner, which was due partly to the hemocoagulation induced by its diuretic effect and may be partly due to the decrease in glomerular filtration rate. OPC-41061 did not affect serum urea nitrogen level in spite of identical hemocoagulation induced. In the case of OPC-41061, the increase in urea.

### TABLE 2

Effects of oral administration of OPC-41061 in combination with furosemide on serum and urinary parameters in conscious male rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
</tr>
<tr>
<td>OPC-41061 (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Furosemide (mg/kg)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

**Urinary excretion rate**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>5</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium (mEq/kg/4 h)</td>
<td>1.30 ± 0.33</td>
<td>1.96 ± 0.35</td>
<td>4.01 ± 0.37**</td>
<td>5.98 ± 0.19</td>
<td>6.12 ± 0.24</td>
<td>6.88 ± 0.24*</td>
</tr>
<tr>
<td>Creatinine (mg/kg/4 h)</td>
<td>4.68 ± 0.42</td>
<td>4.70 ± 0.31</td>
<td>4.91 ± 0.17</td>
<td>4.58 ± 0.16</td>
<td>4.58 ± 0.17</td>
<td>4.71 ± 0.18</td>
</tr>
<tr>
<td>Urea nitrogen (mg/kg/4 h)</td>
<td>30.3 ± 20.2</td>
<td>69.4 ± 18.5</td>
<td>146.8 ± 8.2**</td>
<td>84.9 ± 4.7</td>
<td>73.5 ± 5.6</td>
<td>86.6 ± 7.9</td>
</tr>
<tr>
<td>Electrolyte clearance (ml/kg/4 h)</td>
<td>18.12 ± 3.11</td>
<td>24.50 ± 3.63</td>
<td>40.88 ± 2.62**</td>
<td>60.16 ± 2.17</td>
<td>58.49 ± 2.17</td>
<td>64.48 ± 2.17</td>
</tr>
</tbody>
</table>

**Serum concentration**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4</th>
<th>6</th>
<th>7</th>
<th>5</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea nitrogen (mg/dl)</td>
<td>22.5 ± 1.0</td>
<td>20.2 ± 1.9</td>
<td>24.7 ± 0.9</td>
<td>33.2 ± 0.7</td>
<td>33.0 ± 1.6</td>
<td>36.1 ± 2.0</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.71 ± 0.02</td>
<td>0.85 ± 0.02*</td>
<td>0.73 ± 0.02</td>
<td>0.94 ± 0.03</td>
<td>0.97 ± 0.03</td>
<td>1.07 ± 0.05*</td>
</tr>
</tbody>
</table>

* \( P < .05 \), ** \( P < .01 \), versus administration of furosemide alone.

Author: Hirano et al.

Vol. 292

Page 292
nitrogen excretion may have offset the elevation in serum urea nitrogen.

The balance of sodium and water excretion is important for the treatment of edematous states. That is, sodium excretion is surely necessary to ameliorate ascites and edema, but excess sodium loss may exacerbate pre-existing hyponatremia, which frequently develops in patients with heart failure or liver cirrhosis. Thus, the combination of OPC-41061 and furosemide may be desirable to improve edematous states and hyponatremia simultaneously. In this study, we examined: 1) whether OPC-41061 produces an additive diuretic effect when combined with furosemide, 2) whether OPC-41061 elevates serum osmolality and sodium level even in combination with furosemide, and 3) whether OPC-41061 enhances RAA-system activation by furosemide. When OPC-41061 was administered concomitantly with furosemide, OPC-41061 produced a further increase in urine volume and E-Ch2O and a further decrease in urinary osmolality compared with furosemide alone. Although administration of furosemide alone significantly elevated serum renin activity and aldosterone concentration, concomitant administration of OPC-41061 did not further elevate these parameters. These results suggest that OPC-41061 can produce a further increase in urine volume in addition to furosemide without activating the RAA-system. OPC-41061 dose-dependently elevated serum osmolality and sodium level even when administered in combination with the high dose of furosemide. OPC-41061 could be of utility, alone or in combination with furosemide, for acute correction of fluid/electrolyte disturbances in human disease states.
In conclusion, OPC-41061 alone increased free water excretion and elevated serum osmolality and sodium level. In contrast, furosemide alone markedly increased urinary electrolyte excretion and decreased serum sodium level. The changes in blood hormone levels when OPC-41061 and furosemide were administered alone and in combination reflected the pharmacological activities of the two drugs. Specifically, OPC-41061 increased serum AVP, whereas furosemide increased serum renin and aldosterone levels in addition to serum AVP. It was also confirmed that OPC-41061 produces further diuresis and elevated serum sodium level in the presence of furosemide.

Acknowledgments

We express sincere thanks to Drs. Youichi Yabuuchi and Michiaki Tominaga for encouragement throughout the study; Drs. Hidenori Ogawa, Kazumi Kondo, and Hiroshi Yamashita for their synthetic efforts; Dr. Tominaga for encouragement throughout the study; Drs. Hidenori Ogawa, Kazumi Kondo, and Hiroshi Yamashita for their synthetic efforts; Dr. Tominaga for encouragement throughout the study; and Paul Randolph for checking the manuscript.

References


Paul for checking the manuscript.


