Sildenafil Citrate (Viagra) Enhances Vasodilatation by Atrial Natriuretic Peptide in Normal Dogs

Fuminobu Ishikura, MD; Shintaro Beppu, MD; Toshihiko Asanuma, MD; James B. Seward, MD*; Bijoy K. Khandheria, MD**

Background  Sildenafil citrate (Viagra) is a selective inhibitor of cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type 5 (PDE5) and is indicated for the treatment of erectile dysfunction.1,2 By inhibiting PDE5, sildenafil citrate enhances the normal physiologic action of nitric oxide and cGMP, thereby allowing patients to attain erection adequate for sexual intercourse.1–4 Viagra is contraindicated in patients who may require organic nitrates, such as nitroglycerin patches or sublingual tablets, because the combination use may decrease blood pressure (BP).5,6 Atrial natriuretic peptide (ANP) is reported a potent vasorelaxant and natriuretic agent7,8 and cGMP acts as a second messenger for ANP in the target cell.9,10 Patients with a diagnosis of heart failure (HF) and increased plasma levels of ANP may potentially have some effects with concomitant use of sildenafil citrate.

In this study, we examined the combined effect of sildenafil citrate on hemodynamic changes during infusion of exogenous ANP.

Methods

Animal Preparation

Eighteen healthy beagles weighing 9.8–18 kg (mean ± SD, 13.1±2.2 kg) were used to assess systemic BP, pulmonary artery pressure (PAP), and plasma levels of cGMP. After hemodynamic variables were measured, 0.1lg·kg⁻¹·min⁻¹ of ANP was given during this study. One hour after initiating infusion of ANP, 2 mg/kg of sildenafil citrate or vehicle was given orally via a nasogastric tube. Hemodynamic changes were measured before and 1 h after these administrations. Mean systemic and PAP decreased during infusion of ANP, and further decreased after sildenafil citrate administration, however, mean systemic blood pressure decreased within 10 mmHg. Plasma levels of cGMP also increased after sildenafil citrate administration.

Conclusion  In normal dogs, sildenafil citrate enhances the vasodilator effect of ANP by increasing the cGMP level, however, the concomitant use of sildenafil citrate with ANP will not induce severe hypotension. (Circ J 2007; 71: 1965–1969)

Key Words: Atrial natriuretic factor; Blood pressure; Heart failure

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Effects of ANP in Combination With Sildenafil Citrate

After anesthesia was induced, an infusion of saline was started and urine was collected for 1 h (Fig 1). One hour later, the baseline hemodynamic variables and CO were measured, and blood and urine samples were collected. After the baseline measurements, 0.1 lg·kg⁻¹·min⁻¹ of human ANP (Carperitide, Suntory Co Ltd, Tokyo, Japan) was continuously infused. At 1 h after initiating infusion of ANP, 2 mg/kg of sildenafil citrate dissolved in 20 ml distilled water (sildenafil citrate group, n=12) or only vehicle (vehicle group, n=6) was given via a nasogastric tube. Hemodynamic changes were measured before and 1 h after these administrations. Blood and urine samples were collected every hour.

Systemic vascular resistance (SVR) and total pulmonary resistance (TPR) were derived using standard formulas:
Analysis of Blood and Urine Samples

All blood samples for determining the plasma levels of ANP, cGMP, and sildenafil citrate were collected from an artery. For ANP assay, blood was collected in tubes containing aprotinin (500 kallikrein inhibitory units/ml) and ethylenediaminetetraacetic acid (1 mg/ml) and immediately placed on ice. After centrifugation at 3,000 rpm at 4°C, aliquots of plasma were measured by radioimmunoassay.11 Plasma and urinary concentrations of cGMP were determined by radioimmunoassay with a commercial kit (Yamasa Syoyu Co Ltd, Tokyo, Japan).12 Urine electrolytes, such as sodium and potassium, were measured with an autoanalyzer. The plasma concentration of sildenafil citrate was measured with high-performance liquid chromatography, as described previously.13 As a control, the plasma concentration of sildenafil citrate was measured 1 h after 1 mg/kg of sildenafil citrate was given to 5 unanesthetized dogs.

Statistical Analysis

All values are expressed as mean±SD. A statistical comparison of the plasma concentration of sildenafil citrate was made with the unpaired Student’s t-test. A statistical comparison of hemodynamic parameters and blood and urine sampling data before and after sildenafil citrate or vehicle administration was made with the paired Student’s t-test. A value of p<0.05 was considered statistically significant.

Results

Plasma Concentration of Sildenafil Citrate

One hour after 2 mg/kg of sildenafil citrate was administered, its plasma concentration was 52.8±44.3 ng/ml, and in the 5 conscious dogs at 1 h after 1 mg/kg of the drug was given, the plasma concentration was 155.7±56.0 ng/ml, significantly higher than in the anesthetized dogs.

Baseline Data and Effects of Only ANP Infusion in All Dogs

The baseline data of the sildenafil citrate group and vehicle group showed no significant differences between the groups (Table 1).

Before infusion of exogenous ANP, the plasma level of ANP was 35±17 ng/L. At 1 h after infusion of 0.1 g·kg⁻¹·min⁻¹ of exogenous ANP, the plasma ANP level increased to 485±305 ng/L in all dogs. Mean systemic BP and PAP significantly decreased 1 h after ANP infusion (146±16 vs 137±21 mmHg, 16.8±4.7 vs 14.2±3.9 mmHg, respectively). Mean right atrial pressure and stroke volume (SV) did not change. Heart rate (HR) and CO decreased significantly (155±31 vs 145±26 rpm, 16.8±4.7 vs 14.2±3.9 mmHg, respectively). The plasma level of cGMP increased from 9.1±8.2 to 32.4±21 mmHg. Urine volumes increased significantly from 24.8±13 to 106.5±51.4 ml. The urinary excretion of cGMP, sodium, and potassium also increased significantly (50.0±19.1 vs 129.9±62.6 nmol/h, 3.4±2.4 vs 17.2±7.7 mmol/h, 1.9±1.1 vs 3.3±1.6 mmol/h, respectively).

These changes in the hemodynamic, blood, and urinary values after administration of ANP were almost the same in both groups.

Effects of Sildenafil Citrate With ANP infusion (Table 2)

The plasma level of ANP was unchanged 1 h after sildenafil citrate administration (50±351 vs 534±254 ng/L). Systemic BP and PAP significantly decreased (p<0.001) and mean right atrial pressure did not change 1 h after sildenafil citrate administration. HR, CO, SV, SVR and TPR did not
Sildenafil Citrate Enhances Vasodilatation

Table 2  Hemodynamic, Blood and Urinary Values After Administration of Sildenafil Citrate or Vehicle

<table>
<thead>
<tr>
<th></th>
<th>ANP</th>
<th>ANP + sildenafil</th>
<th>p value</th>
<th>ANP</th>
<th>ANP + vehicle</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systemic blood pressure (mmHg)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>177±27</td>
<td>167±29</td>
<td>&lt;0.001</td>
<td>189±24</td>
<td>187±19</td>
<td>0.41</td>
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<tr>
<td>Diastolic</td>
<td>113±17</td>
<td>108±16</td>
<td>&lt;0.05</td>
<td>124±12</td>
<td>128±10</td>
<td>0.289</td>
</tr>
<tr>
<td>Mean</td>
<td>132±22</td>
<td>126±21</td>
<td>&lt;0.001</td>
<td>146±15</td>
<td>146±14</td>
<td>0.909</td>
</tr>
<tr>
<td><strong>Pulmonary artery pressure (mmHg)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Systolic</td>
<td>23.4±6.5</td>
<td>19.7±5.0</td>
<td>&lt;0.001</td>
<td>25.8±6.8</td>
<td>25.3±6.5</td>
<td>0.295</td>
</tr>
<tr>
<td>Diastolic</td>
<td>7.9±2.7</td>
<td>6.5±3.0</td>
<td>&lt;0.001</td>
<td>6.0±2.1</td>
<td>6.8±1.3</td>
<td>0.224</td>
</tr>
<tr>
<td>Mean</td>
<td>14.2±3.6</td>
<td>11.6±3.2</td>
<td>&lt;0.001</td>
<td>14.2±5.0</td>
<td>14.5±4.8</td>
<td>0.363</td>
</tr>
<tr>
<td><strong>Mean right atrial pressure (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>1.9±1.1</td>
<td>1.8±1.3</td>
<td>0.166</td>
<td>3.0±2.8</td>
<td>3.2±3.3</td>
<td>0.792</td>
</tr>
<tr>
<td><strong>Heart rate (beats/min)</strong></td>
<td>143±28</td>
<td>137±27</td>
<td>0.095</td>
<td>149±22</td>
<td>138±15</td>
<td>0.119</td>
</tr>
<tr>
<td><strong>Cardiac output (L/min)</strong></td>
<td>2.73±0.9</td>
<td>2.39±0.82</td>
<td>0.116</td>
<td>2.34±0.55</td>
<td>2.03±0.54</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Stroke volume (ml)</strong></td>
<td>19.6±8.0</td>
<td>18.1±6.6</td>
<td>0.492</td>
<td>15.9±5.6</td>
<td>14.6±3.7</td>
<td>0.217</td>
</tr>
<tr>
<td><strong>Systemic vascular resistance (mmHg·min/L)</strong></td>
<td>52.6±18.9</td>
<td>56.1±16.8</td>
<td>0.193</td>
<td>64.3±16.8</td>
<td>74.7±21.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Total pulmonary resistance (mmHg·min/L)</strong></td>
<td>5.6±3.88</td>
<td>5.16±1.63</td>
<td>0.073</td>
<td>6.2±2.22</td>
<td>7.35±2.49</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><strong>Plasma ANP (pg/ml)</strong></td>
<td>508±551</td>
<td>534±254</td>
<td>0.788</td>
<td>454±259</td>
<td>465±248</td>
<td>0.864</td>
</tr>
<tr>
<td><strong>Plasma cGMP (pmol/L)</strong></td>
<td>26.5±13.8</td>
<td>46.3±14.5</td>
<td>&lt;0.01</td>
<td>44.2±28.6</td>
<td>44.5±18.6</td>
<td>0.968</td>
</tr>
<tr>
<td><strong>Urine volume (ml)</strong></td>
<td>104±50</td>
<td>136±68</td>
<td>&lt;0.05</td>
<td>111±60</td>
<td>120±64</td>
<td>0.576</td>
</tr>
<tr>
<td><strong>Urine cGMP (mmol/h)</strong></td>
<td>116±47</td>
<td>171±91</td>
<td>&lt;0.01</td>
<td>157±84</td>
<td>127±60</td>
<td>0.232</td>
</tr>
<tr>
<td><strong>Urine sodium (mmol/h)</strong></td>
<td>16.9±7.0</td>
<td>21.4±6.5</td>
<td>&lt;0.05</td>
<td>18.0±9.7</td>
<td>16.7±7.3</td>
<td>0.571</td>
</tr>
<tr>
<td><strong>Urine potassium (mmol/h)</strong></td>
<td>3.0±1.3</td>
<td>3.5±1.2</td>
<td>0.069</td>
<td>3.8±2.1</td>
<td>3.2±1.6</td>
<td>0.192</td>
</tr>
</tbody>
</table>

Abbreviations see in Table 1.
p value: paired t-test.

Fig 2. Serial changes in plasma atrial natriuretic peptide (ANP) levels and cyclic guanosine monophosphate (cGMP) levels. Base, baseline; S, sildenafil citrate; V, vehicle. *ANP vs ANP+S, p<0.01 (paired t-test).

Change 1 h after sildenafil citrate administration.

The cGMP level significantly increased from 26.5±13.8 to 46.3±14.5 nmol/L at 1 h after sildenafil citrate administration. Urine volume and the urinary excretion of cGMP and sodium, increased significantly, however, the urinary excretion of potassium did not change 1 h after sildenafil citrate administration.

Changes in Hemodynamic, Blood and Urine Variables in the Vehicle Group (Table 2)
The plasma level of ANP was unchanged 1 h after vehicle administration (454±259 vs 465±248 ng/L). Systemic BP, PAP, HR and SV did not change, and CO decreased slightly, 1 h after vehicle administration. SVR and TPR slightly but significantly increased 1 h after vehicle administration.

The cGMP level, urine volume and the urinary excretion of cGMP, sodium and potassium did not change 1 h after vehicle administration.

Enhancement of Sildenafil Citrate on Effects of ANP Compared With Vehicle
Sildenafil citrate significantly enhanced the effects of ANP on systemic BP and PAP. Sildenafil citrate also increased plasma cGMP levels at the same plasma ANP levels (Fig 2). Sildenafil citrate also significantly enhanced the effects of ANP on urine volume and the urinary excretion of cGMP and sodium.

Discussion
Sildenafil citrate is a selective inhibitor of cGMP-specific PDE5 and enhances the normal physiologic action of nitric oxide, which induces cGMP production.14–17 By inhibiting PDE5, sildenafil citrate produces smooth muscle relaxation, not only in the corpus cavernosum but also in systemic vascular smooth muscle. For this reason, sildenafil citrate should not be prescribed with organic nitrates, such as nitroglycerin patches or sublingual tablets, because the combination may dramatically decrease BP.5,6 We previ-
ously reported that the combined effect of sildenafil citrate with nitrate resulted in large and protracted decreases in systemic BP and coronary blood flow in coronary arteries with critical stenosis. ANP has been reported to be a potent vasorelaxant that increases cGMP levels in vascular smooth muscle; and this is the first study to report the effect of sildenafil citrate in combination with ANP on the systemic and pulmonary circulation.

**Plasma Concentration of Sildenafil Citrate**

In our study, we chose the dose of 2 mg/kg, which is higher than the recommended clinical dose, because with anesthesia the absorption of sildenafil citrate may deteriorate. In fact, the plasma concentrations 1 h after 2 mg/kg oral administration of sildenafil citrate in the anesthetized dogs was significantly lower than after 1 mg/kg oral administration in unanesthetized dogs. A 2 mg/kg dose of sildenafil citrate for an anesthetized dog, which was not much higher than the clinical dose, still enhanced the effect of ANP on hemodynamic parameters and cGMP levels.

**Effects of Sildenafil Citrate in Combination With ANP on Systemic and Pulmonary Circulation**

Exogenous ANP alone decreased the systemic and the pulmonary BP, as previously reported. Increased plasma ANP levels induced production of cGMP, the intracellular second messenger of ANP. The administration of sildenafil citrate in combination with ANP further decreased systemic and pulmonary BP without changing the plasma level of ANP. Although the plasma level of ANP did not change, the plasma concentration of cGMP increased from 26.5±13.8 to 46.3±14.5 nmol/L in the sildenafil citrate group. However, the plasma concentration of cGMP did not change in the vehicle group. Increased cGMP levels because of administration of sildenafil citrate may further decrease systemic and pulmonary BP, however, mean systemic BP decreased within 10 mmHg, which would not be considered serious in the clinical setting.

Exogenous ANP not only has a vasodilating effect but also causes diuresis, which may decrease the systemic and pulmonary BP. To exclude the effect of hypovolemia, because of pronounced diuresis, on the systemic and pulmonary BP, 100 ml/h of saline was infused in all dogs. The amount of infused saline was almost equal to the volume of urine and thus avoided the effect of diuresis on the hemodynamic parameters.

**Effects of Sildenafil Citrate With ANP on Renal Function**

Exogenous ANP alone increased urine volume and urinary excretion of cGMP, sodium, and potassium, as previously reported. Sildenafil citrate administered in combination with ANP further increased urine volume and urinary excretion of cGMP and sodium without an increase in plasma ANP levels.

Occasionally, ANP produces a rapid and remarkable diuresis that may cause acute dehydration in normal subjects. However, prolonged infusion of ANP does not always increase urine volume. In the present study, the infusion of 100 ml/h of saline may have prevented acute dehydration in the dogs and might explain why urine volume and urinary excretion of cGMP, sodium, and potassium increased after sildenafil citrate administration.

We hypothesized that ANP increased urine volume and urinary excretion of cGMP and sodium through cGMP, and that sildenafil citrate might enhance the effect of ANP on renal function by increasing the cGMP level.

**Effects of Sildenafil Citrate on SVR and TPR**

SVR and TPR are affected by CO. Even if systemic or pulmonary BP decreases, SVR and TPR might increase when CO decreases. In the present study, saline was infused at 100 ml/h in all dogs to exclude the effect of hypovolemia because of pronounced diuresis on systemic and pulmonary BP.

In the vehicle group, SVR and TPR slightly increased. In the sildenafil citrate group, SVR did not increase and TPR tended to decrease slightly. Therefore, sildenafil citrate might overcome the downregulation by ANP of SVR and TPR. ANP hyporesponsiveness is probably multifactorial; but many factors would not have an acute downregulative effect as in the present result. ANP also has sympathoinhibitory effects and increased sympathetic activity is reported to reduce ANP effects at the renal site, as demonstrated by renal denervation. Feng et al reported that the diuretic and natriuretic responses to ANP are significantly increased in congestive HF after presynaptic inhibition of norepinephrine release by low-dose clonidine. Based on those results, sympathetic activity might contribute to early downregulation. This effect seemed to be stronger on the pulmonary circulation than on systemic circulation, and sildenafil citrate was recently reported to improve primary pulmonary hypertension.

**Study Limitations**

We used and measured human ANP instead of dog ANP because the cross-reactivity of human ANP is assumed to be 100% with dog ANP and the amino acid sequences of both are identical. Although we have demonstrated that sildenafil citrate enhances the vasodilatation produced by exogenous ANP, it is unknown whether the same effect occurs with endogenous ANP in a model of HF.

**Clinical Implications**

Plasma ANP concentrations in patients with congestive HF are increased compared with normal subjects. Therefore, sildenafil citrate may enhance the vasodilator effect of ANP in patients with congestive HF; however, this effect is unlikely to be so great as to induce severe hypotension. Indeed, some clinical studies have shown that sildenafil citrate does not induce severe hypotension in the patients with HF.

Tsutamoto et al reported that although the plasma cGMP level correlated with the ANP level in patients with mild congestive HF, this correlation was not found in patients with severe congestive HF, among whom the plasma cGMP levels appeared to reach a plateau despite high levels of plasma ANP. In patients with mild congestive HF who were given exogenous ANP, plasma cGMP levels increased in proportion to those of plasma ANP with saturation. The authors suspected that downregulation of ANP receptors coupled to guanylate cyclase might occur in the peripheral vascular bed of patients with chronic severe congestive HF. Thus, sildenafil citrate may be beneficial in patients with downregulation of ANP binding sites and this effect needs to be studied in an animal model of HF.

**Conclusion**

In normal dogs, sildenafil citrate enhances the vasodilator effect of ANP by increasing cGMP level. The administra-
tion of ANP may induce an additional decrease in systemic or pulmonary BP in patients taking sildenafil citrate, however, the concomitant use of sildenafil citrate with ANP is unlikely to induce severe hypotension.

References