PDE5 Inhibitor Sildenafil Citrate Augments Endothelium-Dependent Vasodilation in Smokers

Masashi Kimura, Yukihiro Higashi, Keiko Hara, Kensuke Noma, Satoshi Sasaki, Keigo Nakagawa, Chikara Goto, Tetsuya Oshima, Masao Yoshizumi, Kazuaki Chayama

Abstract—Smoking is associated with endothelial dysfunction. The purpose of this study was to determine the effect of sildenafil, an inhibitor of phosphodiesterase type 5 (PDE5), on endothelial function in smokers. We evaluated the forearm blood flow (FBF) responses to acetylcholine (ACh), an endothelium-dependent vasodilator, and to sodium nitroprusside (SNP), an endothelium-independent vasodilator, before and after oral sildenafil administration (100 mg) with a strain-gauge plethysmograph in 10 young healthy male smokers and 10 young healthy male nonsmokers. FBF response to ACh was lower in smokers than in nonsmokers. The vasodilatory effects of SNP were similar in both groups. Sildenafil increased the FBF response to ACh from 9.3 ± 2.0 to 12.5 ± 3.5 mL/min per 100 mL tissue in smokers and from 12.6 ± 5.6 to 19.6 ± 8.4 mL/min per 100 mL tissue in nonsmokers, and it increased the response to SNP from 13.3 ± 3.9 to 15.1 ± 4.3 mL/min per 100 mL tissue in smokers and from 14.8 ± 5.2 to 18.4 ± 6.0 mL/min/100 mL tissue in nonsmokers (P < 0.05 for all). The ratio of maximal ACh-stimulated FBF expressed as a ratio of maximal SNP-stimulated FBF significantly increased after administration of sildenafil in both groups. Infusion of Nω-monomethyl-L-arginine, a nitric oxide synthase inhibitor, abolished sildenafil-induced augmentation of the FBF response to ACh in both groups. The findings suggest that endothelial function is impaired in smokers compared with that in nonsmokers, that inhibition of PDE5 by sildenafil significantly increases nitric oxide–mediated vasodilation, and that the activities of PDE5 in smokers and nonsmokers may be similar. (Hypertension. 2003;41:1106-1110.)

Key Words: nitric oxide synthase ■ smoking ■ endothelium ■ blood flow ■ acetylcholine ■ vasodilation

Sildenafil citrate is the first oral agent that has become available for the treatment of erectile dysfunction. Sildenafil is a selective inhibitor of phosphodiesterase type 5 (PDE5) in vascular smooth muscles of the corpus cavernosum and other tissues. Sildenafil blocks the degradation of cGMP, leading to relaxation of vessels by increasing levels of cGMP. Nitric oxide (NO), which is produced from L-arginine in the presence of NO synthase in the endothelium, stimulates cytosolic guanylate cyclase and increases cGMP content in vascular smooth muscle cells, resulting in relaxation of vascular tone.

Cigarette smoking is one of the major cardiovascular risk factors for the development of atherosclerosis.1–2 Several investigators have reported that smoking is associated with endothelial dysfunction.3 Endothelial dysfunction is the initial step in the pathogenesis of atherosclerosis.4–8

It has been shown that zaprinast, a PDE5 inhibitor, enhances endothelium-dependent, NO-mediated vasodilation in isolated rat aortic rings, in intact lamb and cat pulmonary circulation, with experimental pulmonary hypertension, and in cat hindlimb circulation.9–12 Sildenafil has recently been used for treatment of primary or secondary pulmonary hypertension.13 It is thought that the NO-cGMP pathway contributes to the pulmonary artery pressure response to sildenafil. Katz et al14 reported that acute inhibition of PDE5 by sildenafil resulted in increases in endothelium-dependent, flow-mediated vasodilation in patients with chronic heart failure compared with that in the placebo group.

However, there has been no study on the effects of sildenafil on endothelial function in smokers. The purpose of this study was to determine whether sildenafil can restore endothelial function in smokers.

Methods

Subjects

The subjects were 10 young healthy male smokers (mean age, 28.1 ± 3.9 years) and 10 young healthy male nonsmokers (mean age, 28.3 ± 3.6 years) who had no history of cardiovascular disease, hypertension, or other diseases. All of the smokers had smoked >20 cigarettes per day for >10 years. The Ethics Committee of Hiroshima University Faculty of Medicine approved the study protocol. Informed consent for participation in the study was obtained from all subjects.
pressure were measured at 1 hour after oral administration of sildenafil or placebo. The effects of ACh and SNP were determined again by the same method as that used before sildenafil or placebo administration. If the effect of ACh was determined first, the effect of SNP was determined 1 week later by the same methods.

After a 30-minute rest period, N\(^{\text{G}}\)-monomethyl-L-arginine (L-NMMA, Clinalfa Co), an NO synthase inhibitor, was infused intra-arterially at a dose of 8 \(\mu\)g/min for 5 minutes while the baseline FBF and arterial blood pressure were recorded, and ACh (3.75, 7.5, and 15 \(\mu\)g/min) was administered.

## Results

### Clinical Characteristics

Baseline clinical characteristics in 10 smokers and 10 nonsmokers before and after sildenafil administration are summarized in the Table. All parameters before and after sildenafil or placebo administration were similar in both groups. Sildenafil or placebo did not alter systemic hemodynamics such as blood pressure, heart rate, and lipid profile.

### Effects of ACh and SNP on Baseline FBF in Smokers and Nonsmokers

There was no significant difference between baseline FBF in smokers and that in nonsmokers. FBF response to ACh was lower in smokers than in nonsmokers (9.3±2.0 versus 12.6±5.6 mL/min per 100 mL tissue, \(P<0.05\); Figure 1). There was no significant difference between the responses to SNP in smokers and nonsmokers (14.8±5.2 versus 13.3±3.9 mL/min per 100 mL tissue, \(P=\text{NS}\); Figure 1).

### Study Protocol

None of the subjects received any drugs for at least 24 hours before the start of the study. Vasodilatory responses were evaluated before and after administration of 100 mg sildenafil (Viagra; Pfizer Pharmaceutical Co) or placebo. Forearm vascular responses to acetylcholine (ACh, Daiichi Pharmaceutical Co) and to sodium nitroprusside (SNP, Maruishi Pharma Co) were evaluated before and after sildenafil administration. The effects of ACh and SNP were determined on 2 separate occasions in 10 subjects. Baseline FBF and arterial blood pressure were measured. Then the infusions of the endothelium-dependent vasodilator ACh (3.75, 7.5, and 15 \(\mu\)g/min) or the endothelium-independent vasodilator SNP (0.75, 1.5, and 3.0 \(\mu\)g/min) were performed randomly every 5 minutes. FBF was measured with a mercury-filled Silastic strain-gauge plethysmograph, as previously described.15–17 FBF during the final 2 minutes of each infusion was measured. Each study proceeded after the FBF had returned to baseline level. The FBF was calculated by 2 independent observers blinded to the study protocol from the linear portions of plethysmographic recordings. The intraobserver coefficient of variation was 3.0%. We confirmed the reproducibility of FBF responses to ACh and SNP on 2 separate occasions in 10 healthy men (mean age, 24±4 years). The coefficients of variation were 6.2% and 4.6%, respectively.

After a 30-minute rest period, 100 mg sildenafil or placebo was administered orally to each subject. Baseline FBF and arterial blood pressure were measured at 1 hour after oral administration of sildenafil or placebo. The effects of ACh and SNP were determined again by the same method as that used before sildenafil or placebo administration. If the effect of ACh was determined first, the effect of SNP was determined 1 week later by the same methods.

After a 30-minute rest period, N\(^{\text{G}}\)-monomethyl-L-arginine (L-NMMA, Clinalfa Co), an NO synthase inhibitor, was infused intra-arterially at a dose of 8 \(\mu\)g/min for 5 minutes while the baseline FBF and arterial blood pressure were recorded, and ACh (3.75, 7.5, and 15 \(\mu\)g/min) was administered.

### Clinical Characteristics of Smokers and Nonsmokers Before and After Sildenafil Administration

<table>
<thead>
<tr>
<th>Variables</th>
<th>Smokers (n=10)</th>
<th>Nonsmokers (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>22.3±3.0</td>
<td>23.1±4.1</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>120.4±11.1</td>
<td>125.8±11.7</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>63.5±9.0</td>
<td>63.4±8.5</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>68.2±6.2</td>
<td>69.1±7.5</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>4.86±0.70</td>
<td>4.39±0.87</td>
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<tr>
<td>Triglyceride, mmol/L</td>
<td>1.37±0.19</td>
<td>1.14±0.74</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.29±0.19</td>
<td>1.15±0.24</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>2.94±0.75</td>
<td>2.47±1.06</td>
</tr>
<tr>
<td>FBF, mL/min per 100 mL tissue</td>
<td>5.0±0.8</td>
<td>6.3±1.8*</td>
</tr>
</tbody>
</table>

All results are presented as mean±SD. HDL indicates high density lipoprotein; LDL, low density lipoprotein; FBF, forearm blood flow. *\(P<0.05\) vs before sildenafil administration.
Effects of ACh and SNP on FBF After Sildenafil or Placebo Administration in Smokers and Nonsmokers

Sildenafil significantly increased baseline FBF in both groups (from 5.0±0.7 to 6.3±1.7 in smokers and from 4.9±1.5 to 8.4±2.3 in nonsmokers, *P*<0.05, Table). The FBF responses to ACh and to SNP after sildenafil administration were significantly higher than those before sildenafil administration of each dose in both groups (Figures 2 and 3). Placebo did not alter baseline FBF and the FBF responses to ACh and to SNP in both groups (Figures 2 and 3).

The ratio of maximal ACh-stimulated FBF expressed as a ratio of maximal SNP-stimulated FBF significantly increased after sildenafil administration in both groups (from 0.73±0.21 to 0.88±0.21 in smokers and from 0.73±0.44 to 0.84±0.27 in nonsmokers, *P*<0.05, Figure 4). The ratio of maximal ACh-stimulated FBF expressed as a ratio of maximal SNP-stimulated FBF after placebo administration in both groups were similar (from 0.75±0.23 to 0.71±0.24 in smokers and from 0.77±0.35 to 0.74±0.29 in nonsmokers). Neither arterial blood pressure nor heart rate was significantly changed by intra-arterial infusion of either ACh or SNP in both groups.

**Effects of ACh on FBF in the Presence of L-NMMA After Sildenafil Administration in Smokers and Nonsmokers**

Intra-arterial infusion of L-NMMA reduced the baseline FBF after sildenafil administration and abolished sildenafil-induced augmentation of the FBF response to ACh in both groups (smokers, 12.5±3.5 versus 10.4±2.3 mL/min per 100 mL tissue, *P*<0.05; nonsmokers, 12.6±5.6 versus 15.6±7.4 mL/min per 100 mL tissue, *P*<0.05; Figure 5). In addition, the FBF responses to ACh before sildenafil administration and after sildenafil administration were similar in subjects treated with intra-arterial infusion of L-NMMA in both groups (smokers, 9.3±2.0 versus 10.4±2.3 mL/min per 100 mL tissue, *P*NS; nonsmokers, 12.6±5.6 versus 15.6±7.4 mL/min per 100 mL tissue, *P*NS; Figure 5). Neither arterial blood pressure nor heart rate was significantly changed by intra-arterial infusion of ACh in the presence of L-NMMA in both groups.

**Discussion**

In the present study, endothelial function was impaired in smokers compared with that in nonsmokers. Oral administration of sildenafil caused vasodilation of forearm resistance arteries and increased both endothelium-dependent vasodilatory response to ACh and endothelium-independent vasodilatory response to SNP in smokers and nonsmokers. The ratio of maximal ACh-stimulated FBF expressed as a ratio of maximal SNP-stimulated FBF significantly increased after sildenafil administration, and the ratios in smokers and nonsmokers were similar. In addition, L-NMMA abolished the sildenafil-induced augmentation of FBF response to ACh. These findings suggested that inhibition of PDE5 by sildenafil significantly increased NO-mediated vasodilation and that the activities of PDE5 in smokers and nonsmokers may be similar.

We selected healthy and young men (mean age, 28.1±3.4 years; range, 22 to 32 years) to eliminate the possibility of alteration in endothelial function caused by factors such as hypertension, heart failure, atherosclerosis, hypercholesterolemia, diabetes mellitus, and aging. FBF response to ACh was significantly lower in smokers than in nonsmokers, whereas...
the FBF responses to SNP in the 2 groups were similar. Our results are consistent with those of previous studies indicating that endothelium-dependent vasodilation is impaired in smokers compared to nonsmokers. It is generally accepted that endothelium-dependent vasodilation is selectively impaired in smokers.

Acute oral administration of sildenafil, a PDE5 inhibitor, significantly increased baseline FBF in both smokers and nonsmokers. This vasodilatory effect of sildenafil may be due to inhibition of PDE5 activity, resulting in inhibition of the degradation of cGMP in vascular smooth muscle cells. Jackson et al reported that intra-arterial infusion of sildenafil caused a modest vasodilation of resistance arteries in healthy men. These findings suggest that sildenafil per se has vasodilatory effects on resistance arteries in humans.

Sildenafil significantly increased the FBF responses to ACh and SNP in both smokers and nonsmokers. The ratio of maximal ACh-stimulated FBF expressed as a ratio of maximal SNP-stimulated FBF significantly increased after sildenafil administration in both groups, indicating that sildenafil may predominately enhance endothelium-dependent vasodilation in smokers and nonsmokers through an increase in NO availability. Aydin et al investigated the effect of sildenafil together with the effects of SNP and ACh and also investigated the mechanism by which sildenafil acts in vitro by using isolated strips of rabbit corpus cavernosum. Sildenafil enhanced the relaxing effects of SNP and ACh on the phenylephrine-induced contraction of rabbit cavernosal tissue. Katz et al found a significant increase in flow-mediated dilation in the brachial artery after administration of sildenafil compared with that after administration of a placebo in ambulatory patients with chronic heart failure, and they reported that inhibition of PDE5 by sildenafil acutely improves endothelium-dependent vasodilation in patients with heart failure. These results support our findings. In contrast, Dishy et al reported that sildenafil increased sensitivity to nitroglycerin, an exogenous NO donor, by ~4-fold but did not affect endothelium-dependent, NO-mediated responses in either the hand or forearm vasculature in healthy men. Although the precise reason for this discrepancy is not known, it may be due to the difference in doses of sildenafil used or to the difference in the methods of assessing endothelial function.

Intra-arterial infusion of L-NMMA, an inhibitor of NO synthase, reduced the baseline FBF after sildenafil administration and abolished sildenafil-induced augmentation of the FBF response to ACh in both smokers and nonsmokers. In addition, the FBF responses to ACh before sildenafil and after sildenafil administration were similar in subjects treated with intra-arterial infusion of L-NMMA. These findings also indicate that sildenafil may improve endothelium-dependent vasodilation through increased NO production.

Eleven families of PDEs have been identified. PDEs differ in their primary structure, tissue distribution, affinities for cyclic nucleotides, and sensitivities to Ca and various inhibitors. PDE5 is highly specific for cGMP and is expressed in skeletal, cardiac, and smooth muscle. The molecular
mechanisms underlying the regulation of PDE5 activity, particularly that in intact tissue, are not clear. It is known, however, that the activity of PDE5 is regulated mainly by phosphorylation by PKG (a cGMP-dependent protein kinase).21,22 We suspected that there are differences between PDE5 activities in smokers and nonsmokers. However, the results of the present study showed that there was no significant difference between the FBF response to SNP after sildenafil administration in smokers and nonsmokers and suggested that the activities of PDE5 in smokers and nonsmokers may be similar.

In conclusion, sildenafil may predominately enhance endothelium-dependent vasodilation in both smokers and nonsmokers through an increase in NO availability. No significant difference was found between activities in smokers and nonsmokers. Endothelial dysfunction is an initial step in arteriosclerosis, leading to increases in the incidences of cardiovascular and cerebrovascular diseases. Therefore, it is clinically important to show that some interventions restore endothelial function. Sildenafil per se may be useful for improving endothelial function as a new treatment for atherosclerosis. Further studies on the effects of sildenafil in patients with essential hypertension, heart failure, diabetes mellitus, and other cardiovascular diseases in which endothelium-dependent vasodilation is impaired are needed.

References


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