Interaction of Sildenafil With cAMP-Mediated Vasodilation In Vivo

Christoph Schalcher, Karin Schad, Hans Peter Brunner-La Rocca, Ruth Schindler, Erwin Oechslin, Christoph Scharf, Gabor Suetsch, Osmund Bertel, Wolfgang Kiowski

Abstract—Sildenafil inhibits cGMP breakdown by phosphodiesterase 5. In vitro, increased cGMP levels inhibit cAMP breakdown by phosphodiesterase 3. It is uncertain, however, whether sildenafil increases biological effects of interventions increasing cAMP levels in vivo. The objective of the present study in 40 healthy male volunteers was to determine the existence and extent of interactions with sildenafil and vasodilators acting via cGMP or cAMP or independently from these mediators on the arterial tone of the human forearm. Forearm blood flow (FBF) responses (plethysmography) to brachial artery infusions of 3 doses each of nitroglycerin, which increases cGMP levels; of isoprenaline and milrinone, which increase cAMP levels; and of verapamil as a control were assessed at baseline and 80 minutes after 50 mg oral sildenafil in 10 volunteers each. Sildenafil increased FBF (2.5±0.1 to 3.5±0.2 mL/min per 100 mL, P<0.001; n=40). At equipotent vasodilator dosages, sildenafil increased FBF from 7.5±1.0 to 9.8±1.2 mL/min per 100 mL for nitroglycerin, from 8.3±1.0 to 10.4±1.4 mL/min per 100 mL for isoprenaline, and from 8.1±1.0 to 10.3±1.2 mL/min per 100 mL for milrinone and slightly decreased FBF from 7.7±1.3 to 7.1±1.2 mL/min per 100 mL for verapamil. ANOVA for repeated measures revealed a significant interaction between sildenafil and the type of vasodilator on FBF (P<0.01). The responses of FBF to nitroglycerin, milrinone, and isoprenaline after sildenafil were similarly increased compared with the response to verapamil (P<0.01). Sildenafil markedly enhanced the arterial vasodilator response to nitroglycerin, milrinone, and isoprenaline. The response to milrinone and isoprenaline is compatible with an interaction between cGMP and phosphodiesterase 3 or an enhancement of the NO component of cAMP-mediated vasodilation, and raises the possibility of enhanced biological effects of interventions leading to increases of cAMP in the presence of sildenafil. (Hypertension. 2002;40:686–690)

Key Words: cyclic GMP ■ cyclic AMP ■ muscle, smooth, vascular ■ human

Release of NO by penile nerve endings stimulates guanylate cyclase and thus increases smooth muscle cGMP production in the corpus cavernosum. This results in smooth muscle relaxation of penile sinusoids, increased inflow of blood, and ultimately erection.1–3 By inhibiting cGMP-specific phosphodiesterase 5, which is the major phosphodiesterase isoform in the human corpus cavernosum,4 sildenafil compensates for reduced NO release and cGMP production and for impairment of penile perfusion in male erectile dysfunction.4–10 Because of the presence of phosphodiesterase 5 in vascular tissue other than the corpus cavernosum,11,12 systemic side effects of sildenafil were to be anticipated. Despite the good safety profile of sildenafil in phase II and III trials in >3000 patients,13,14 an excess mortality was observed in comparison with locally applied agents in postmarketing analysis, reflecting less stringent prescription of the drug in clinical practice outside of controlled studies.15,16 Lowering blood pressure in combination with physical effort during the sexual act could seriously impair organ perfusion in patients suffering from cardiovascular diseases. Also, the potentially harmful interaction of sildenafil with NO donors such as nitroglycerin, which are expected to be used by a significant proportion of such patients, may further accentuate the problem.17,18 Interactions of sildenafil with drugs other than NO donors are also possible. Elevated cGMP levels modulate the activity of other phosphodiesterase isoforms (eg, phosphodiesterase 3) in vitro.12,19,20 However, the biological importance of the modulatory influence of sildenafil-induced increases of cGMP on phosphodiesterase isoforms other than phosphodiesterase 5 remains to be determined in vivo. Thus, the aim of the present study was to compare the interaction of sildenafil with vasodilators acting through either cGMP or cAMP in comparison with a positive control (ie, a calcium channel blocker) not involving cGMP or cAMP.
Methods

Subjects
Forty healthy male volunteers with a mean age of 28±0.7 years participated in the examination. They were recruited by an advertisement, and written informed consent was obtained before inclusion into the study, which was approved by the hospital’s ethics committee.

Measurement of Forearm Blood Flow
Forearm blood flow (FBF) was measured by means of venous occlusion plethysmography as described previously.23,24 In brief, mercury-in-silastic strain gauges were placed on both forearms and coupled to electronically calibrated plethysmographs (Hokanson, EC4). Venous occlusion pressure (40 mm Hg) was achieved by a rapid inflator (Hokanson, EC10) connected to blood pressure cuffs placed proximally to the subjects’ elbows. All circulation to the hand was arrested 1 minute before and during the forearm measurements by suprasystolic inflation of a pediatric cuff on the wrist. FBF was calculated from the linear portion of the plethysmographic recording by the use of a digitizing board and computer program. For statistical evaluation, the mean from 4 recordings obtained within 1 minute was used.

Study Protocol
FBF responses to brachial artery infusion of nitroglycerin (prepared by the hospital’s pharmacy), milrinone (Corotrop, Sanofi Winthrop), isoprenaline (Isuprel, Abbott), or verapamil (Isoptin, Knoll) at baseline and 80 minutes after oral administration of 50 mg sildenafil (a gift from Pfizer Inc, Züri, Switzerland) were assessed in 10 healthy volunteers each. The studies began at 8:00 AM, with the subjects resting and fasting in a temperature-controlled room. The subjects had refrained from smoking and ingestion of beverages or food containing caffeine for at least 12 hours before the study. No subjects were taking any medication regularly. A 23-gauge polyethylene catheter was inserted into the brachial artery of the nondominant arm under local anesthesia for infusion of vasodilators and recording of arterial pressure. Thirty minutes after completion of instrumentation, basal FBF was recorded. Next, one of the vasodilators was infused in 3 incremental dosages for 4 minutes each (nitroglycerin: 0.0313, 0.0625, and 0.125 μg/min per 100 mL tissue; isoprenaline: 2.2, 4.0, and 12.0 μg/min per 100 mL tissue; and verapamil: 5.15, and 40 μg/min per 100 mL tissue). During the last minute of each infusion, plethysmographic measurements were obtained. After cessation of the infusion, FBF, heart rate, and arterial blood pressure were measured in 10-minute intervals. One hour after cessation of infusion, the subjects received 50 mg of sildenafil orally. After another 80 minutes, vasodilator infusions were repeated in an identical fashion.

Statistical Analysis
Statistical analysis was performed by use of the SPSS statistical software package, version 9.0 (SPSS Inc). Results are represented as mean±SEM. Because all variables were normally distributed, parametric tests were used. General linear model for repeated measures was used to test the responses to the infusions in the 4 groups, to compare the responses before and after intake of sildenafil, and to test differences in the response to sildenafil between the different groups. Bonferroni’s adjustment for multiple comparisons was used when appropriate. A value of P<0.05 was considered to indicate a statistically significant difference.

Results
Hemodynamics are presented in the Table. The most notable effects of sildenafil were increases in both heart rate and arterial pressure, which were not different among the 4 groups (P>0.1). Figure 1 shows FBF of the noninstrumented control arm during the experiment. A slight degree of spontaneous vasodilation was observed over time (2.4% per 10 minutes), which was independent of the effect of sildenafil. In addition, sildenafil caused a significant increase in FBF by 34% (P<0.001) and a reduction of calculated forearm vascular resistance from 48.8±3.9 to 38.2±4.2 arbitrary units (P<0.01). A similar degree of vasodilation was noted in the instrumented arm, in which blood flow changed from 2.5±0.1 mL/min per 100 mL at baseline to 3.5±0.2 mL/min per 100 mL 80 minutes after sildenafil (P<0.001).

As depicted in Figure 2, brachial artery infusions of all drugs induced dose-dependent increases in FBF (all, P<0.001), and the vasodilatory responses to nitroglycerin, milrinone, and isoprenaline but not verapamil were significantly enhanced by sildenafil. In multivariate analysis, the

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>80 Minutes After Sil</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>86.8±1.3</td>
<td>88.8±1.2</td>
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<td>Systolic blood pressure, mm Hg</td>
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<td>124.7±1.3</td>
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<td>Diastolic blood pressure, mm Hg</td>
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<td>69.5±1.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>54.0±1.1</td>
<td>55.2±0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>58.8±1.6</td>
<td>63.6±1.5</td>
<td>0.004</td>
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</tbody>
</table>

Values are mean±SEM; n=40. Sil indicates sildenafil.
interaction between sildenafil and the vasodilatory response to nitroglycerin, milrinone, and isoprenaline was significantly different from that to verapamil (\( P = 0.01 \)). In contrast, this interaction did not differ between the groups receiving nitroglycerin, milrinone, and isoprenaline (\( P > 0.1 \)).

As depicted in Figure 2, baseline FBF was higher after sildenafil except in subjects assigned to verapamil. To account for the differences in baseline values, changes of flow were also analyzed. As shown in Figure 3, results for nitroglycerine and isoproterenol were similar when analyzed in this way, but milrinone showed only a nonsignificant increase after sildenafil. Importantly, the curves for nitroglycerine, isoproterenol, and milrinone separated increasingly for higher doses, a finding that argues against an important effect of increased baseline flow.

Because the maximal vasodilatory responses to the 4 vasodilators were different (verapamil is greater than isoprenaline is greater than nitroglycerin, which is equal to milrinone; \( P < 0.001 \)), analysis was repeated selecting doses leading to comparable absolute FBF levels before sildenafil intake (ie, highest dose for nitroglycerin [7.3 ± 1.0 mL/min per 100 mL] and milrinone [8.1 ± 1.0 mL/min per 100 mL], middle dose for isoprenaline [8.3 ± 1.0 mL/min per 100 mL], and lowest dose for verapamil [7.7 ± 1.3 mL/min per 100 mL], \( P > 0.1 \) between groups]). Again, the vasodilatory response was significantly enhanced by sildenafil for nitroglycerin, isoprenaline, and milrinone (\( P < 0.05 \)) but remained unchanged for verapamil (\( P < 0.05 \) between verapamil and other groups in multivariate analysis) (Figure 4).

**Discussion**

In this study of normal volunteers, oral intake of a submaximal dose of sildenafil caused substantial arterial vasodilation as shown by the increase in FBF, whereas blood pressure showed small changes only. This vasodilator effect was accompanied by an increase in heart rate, suggesting baroreflex activation and sympathetic stimulation to a degree sufficient to maintain recumbent arterial pressure via increased cardiac output in these young healthy volunteers. Phosphodiesterase 5 is the predominant phosphodiesterase isoenzyme in corpus cavernosum, but it is also widely distributed in vascular tissue. Thus, sildenafil has the potential of exerting direct arterial and venous vasodilator effects via inhibition of vascular phosphodiesterase 5, and the observed vasodilation fits well with its known pharmacological profile. Interestingly, sildenafil enhanced the expected vasodilator response not only to a cGMP-dependent vasodilator, ie, nitroglycerin, but also to the cAMP-dependent vasodilators isoprenaline and milrinone. This was in contrast to vasodilation induced by the calcium channel blocker verapamil, which was not significantly influenced by sildenafil. These findings, therefore, suggest that the phosphodiesterase 5 inhibitor sildenafil enhances the biological effects not only of interventions leading to increased cGMP levels but also of interventions leading to increased cAMP levels.

The interaction of sildenafil with nitroglycerine was expected because of the mechanism of action of sildenafil. During blockade of cGMP breakdown by sildenafil, exogenous NO donors bypass autoregulation of vascular NO release, resulting in increased and potentially uncontrolled vasodilation. This interaction between sildenafil and NO donors has previously been demonstrated both in vitro and in vivo. Thus, rabbit aortic rings showed amplification of relaxation in the presence of sildenafil and nitroglycerin. In studies in healthy volunteers, the blood pressure reduction during administration of nitroglycerin was potentiated in the presence of sildenafil.

The interaction with vasodilators acting through cAMP, however, was less expected. In the present study, sildenafil significantly enhanced the vasodilator response to 2 cAMP-dependent vasodilators, which increase cAMP by entirely different means. Thus, vasodilation was enhanced with isoprenaline, which stimulates adenylyl cyclase and thereby increases cAMP, and with milrinone, which inhibits phosphodiesterase 3 and thus the breakdown of cAMP. Previous studies investigating interactions of sildenafil with cAMP-mediated effects are controversial. Although in vitro studies in corpus cavernosum tissue and in dog coronary arteries
demonstrated no increase of tissue cAMP by sildenafil, even in the presence of NO-donors. Recent work has shown a modest increase of cAMP in both human cardiac ventricle and corpus cavernosum tissue in vitro in the presence of sildenafil-induced cGMP elevation. Moreover, and possibly more relevant to the present data, coronary flow reserve in patients with coronary artery disease, assessed by intracoronary application of adenosine (another vasodilator acting through cAMP), was enhanced in the presence of sildenafil.

The apparent interaction of sildenafil with vasodilators acting through cAMP to a similar extent as with NO donors is an intriguing finding. Vascular phosphodiesterases are differentially distributed in endothelial cells and vascular smooth muscle cells, and there is evidence of interactions between cGMP and cAMP degrading enzymes both in vascular endothelial and smooth muscle cells. For instance, phosphodiesterase 3, which is present in vascular smooth muscle, is inhibited by cGMP. Thus, the vasodilator effects of sildenafil might partially be owing to phosphodiesterase 3 inhibition, an effect possibly potentiated in the presence of inhibitors of cAMP breakdown, such as milrinone, or agents increasing cellular cAMP levels, such as isoprenaline. Sildenafil may significantly increase plasma cGMP levels. This increase in cGMP raises the possibility of modulation of cGMP-regulated phosphodiesterases in tissues in which phosphodiesterase 5 is not present (eg, the myocardium).

An alternative explanation for our findings with isoprenaline would be in the observation that β-adrenergic vasodilation is partly mediated through NO. Thus, removal of endothelium strongly diminishes the vasodilator response to isoproterenol in vitro. In humans, forearm vasodilation to isoproterenol is markedly attenuated by coadministration of NO synthase inhibiting L-arginine analogues. Although the intracellular signaling pathways that couple β-adrenoceptor stimulation to NO synthase are not entirely clear, it has been suggested that cAMP acts as a second messenger. Thus, the enhanced response to milrinone after administration of sildenafil could also be explained by blockade of NO degradation. Coadministration of a NO synthase inhibitor would be required to solve this issue.

No significant interaction between sildenafil and the calcium channel blocker verapamil was observed, arguing against an unspecific vasodilatory effect of sildenafil. However, an additive hypotensive effect of sildenafil and the calcium channel blocker amlodipine was found in older hypertensive subjects. Several reasons may account for this difference. Stimulation of the sympathetic nervous system was observed in our study group, as indicated by the increase in heart rate, which, however, was similar in the 4 groups. Consistent with our results, a significant increase of microelectrographically assessed sympathetic nerve activity was found after 100 mg of oral sildenafil in normal volunteers. Baroreflex stimulation may be blunted in older hypertensives in comparison to healthy volunteers, reflected by the relatively small increase in heart rate by only 2.1 bpm in patients. Also, 100 mg of sildenafil was given in that study compared with 50 mg in the present study, which is the recommended maximal starting dose. Finally, other studies in normal volunteers showed that only higher doses of sildenafil decreased blood pressure.

**Study Limitations**

Some limitations apply to this study. The human forearm model, although widely used to study pharmacological drug effects, may not be representative for other vascular beds, as tissue distribution of the different phosphodiesterases varies. Thus, results obtained in this predominantly skeletal muscle vascular bed need not necessarily reflect responses of other vascular beds, although a comparable interaction was found in the coronary circulation.

The vasodilators used in our study were not applied in equipotent dosage steps, making comparison of entire dose response curves somewhat difficult. However, results at comparable degrees of vasodilation yielded the same results as the analysis of complete titration curves of the vasodilators. Sildenafil increased FBF, and repeat dose response curves for the various vasodilators started from significantly higher levels compared with control experiments before sildenafil, except for subjects assigned to verapamil infusions. This poses a potential problem in interpreting the results. However, both the absolute FBF levels and the increase from the respective control values were significantly greater after nitroglycerin and isoproterenol. Importantly, dose response curves for nitroglycerin, isoproterenol, and milrinone separated more at higher dosages. This would not be expected if sildenafil only had an additive effect. Moreover, the overall vasodilator response to nitroglycerin, isoproterenol, and milrinone was significantly different from that observed with verapamil.

Also, only 1 vasoactive drug was tested in an individual. Intraindividual comparison of drug effects usually are preferable to group comparisons, but the long duration of the studies prevented multiple drug administrations during the same session, whereas the invasive nature of the experiments prevented repeated studies on different days.

Finally, results obtained in a population of young healthy male subjects may not be directly applied to elderly patients suffering from cardiovascular disease.

**Perspectives**

Our findings suggest that sildenafil, in addition to the expected enhancement of cGMP-mediated vascular effects, also enhances cAMP-mediated effects. Whether this is owing to an interaction of sildenafil with phosphodiesterase 3 or relates to the fact that β-adrenergic and cAMP-mediated vasodilation partly depend on NO release remains to be elucidated. Irrespective of the potential mechanism(s) underlying this observation, it is important to recall that a variety of drugs in clinical medicine act through cAMP. Most prominent among them are various sympathomimetic drugs used in the treatment of asthma and for cardiovascular support in emergency situations. Importantly, the vasodilating effects may be further enhanced by the concomitant use of theophylline, also given to asthmatic patients. Additionally, dipyridamole (increasing both cGMP and cAMP), but also some herbal therapies, may be candidates for interactions with sildenafil. Conceivably, adverse effects of sildenafil might be enhanced...
when administered together with these drugs. Clearly, whether such interactions exist and whether they are of clinical relevance needs further study.

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