Sildenafil Prevents Endothelial Dysfunction Induced by Ischemia and Reperfusion via Opening of Adenosine Triphosphate–Sensitive Potassium Channels

A Human In Vivo Study

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Background—Animal studies have demonstrated that administration of sildenafil can limit myocardial damage induced by prolonged ischemia, an effect that appears to be mediated by opening of adenosine triphosphate–sensitive potassium (K_{ATP}) channels. No study has investigated whether sildenafil can also prevent the impairment in endothelium-dependent vasodilatation induced by ischemia-reperfusion (IR) in humans.

Methods and Results—In a double-blind, placebo-controlled, crossover design, 10 healthy male volunteers (25 to 45 years old) were randomized to oral sildenafil (50 mg) or placebo. Two hours later, endothelium-dependent, flow-mediated dilatation (FMD) of the radial artery was measured before and after IR (15 minutes of ischemia at the level of the brachial artery followed by 15 minutes of reperfusion). Seven days later, subjects received the other treatment (ie, placebo or sildenafil) and underwent the same protocol. Pre-IR radial artery diameter and FMD, as well as baseline radial artery diameter after IR, were similar between visits (P=NS). After placebo administration, IR significantly blunted FMD (before IR: 7.9±1.1%; after IR: 1.2±0.7%, $P<0.01$). Importantly, sildenafil limited this impairment in endothelium-dependent vasodilatation (before IR: 7.0±0.9%; after IR: 6.2±1.1%, P=NS; $P<0.01$ compared with placebo). In a separate protocol, this protective effect was completely prevented by previous administration of the sulfonylurea glibenclamide (glyburide, 5 mg), a blocker of K_{ATP} channels (n=7; FMD before IR: 10.3±1.5%; after IR: 1.3±1.4%, $P<0.05$).

Conclusions—In humans, oral sildenafil induces potent protection against IR-induced endothelial dysfunction through opening of K_{ATP} channels. Further studies are needed to test the potential clinical implications of this finding. **(*Circulation*. 2005;111:742-746.)**

Key Words: endothelium ■ ischemia ■ reperfusion

Multiple lines of evidence have emphasized the importance of the vascular endothelium in regulating vasomotor, thrombotic, and inflammatory mechanisms that are critical in the pathophysiology of tissue injury induced by ischemia and reperfusion (IR).[1] Endothelial cells appear to be more sensitive to IR than myocytes, and during ischemia, a state of reduced endothelial responsiveness to specific stimuli ("endothelial dysfunction") temporally precedes (and contributes to) the appearance of IR-induced tissue necrosis.[2,3] Importantly, studies have demonstrated that exposure to brief periods of ischemia (ie, ischemic preconditioning) and/or to specific pharmacological stimuli can modulate myocardial sensitivity to IR-induced injury.[4] To date, although evidence exists that a phenomenon analogous to ischemic preconditioning can be elicited at the level of the endothelium,[3] studies have not investigated whether a similar protective state can also be induced pharmacologically, in vivo, in humans.

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Sildenafil citrate is a highly specific inhibitor of type V phosphodiesterase, an enzyme responsible for the catabolism of cGMP in multiple vascular districts.[5] Although currently marketed for the treatment of erectile dysfunction, sildenafil has beneficial effects in other cardiovascular conditions, including congestive heart failure and pulmonary hypertension as well as in the setting of endothelial dysfunction.[6,7] Recent studies show that sildenafil administration enhances endothelium-dependent vasodilatation and exercise tolerance in patients with coronary artery disease,[8,9] and animal studies have also documented that sildenafil induces potent cardiac

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protection against IR.[10,11] Although there continues to be some controversy concerning these observations,[12] evidence that sildenafil induces cardioprotection is consistent with research reporting that both nitric oxide and nitric oxide–derived cGMP can induce the same state of protection via opening of adenosine triphosphate–sensitive potassium (K_{ATP}) channels.[13–15] Interestingly, although the majority of research in this area has involved (cardio)myocytes, multiple lines of evidence suggest that stimuli leading to K_{ATP} channel opening can induce a potent protective effect against IR in different cell types, and recent studies have confirmed that similar mechanisms can also modulate the endothelial response to IR. For example, animal models have documented that the administration of specific openers of K_{ATP} channels leads to a state of reduced susceptibility to IR injury in the endothelium, whereas blockade of these channels with sulfonylureas inhibits endothelial cell ischemic preconditioning.[16] The role of K_{ATP} channels has been investigated less extensively in humans.

In a human in vivo model of IR-induced endothelial dysfunction, we set out to test (1) whether sildenafil administration can prevent the impairment in endothelium-dependent vasodilatation induced by IR and (2) whether this effect is mediated by K_{ATP} channel opening.

Methods

The Ethics Committee of the University of Siena, Italy, approved the study. Written informed consent was obtained in all cases. Studies were conducted in a quiet, temperature- and humidity-controlled environment.

Protocol 1: Effect of Sildenafil

Ten healthy male nonsmoking volunteers (25 to 45 years old) were enrolled in this double-blind, randomized, placebo-controlled crossover trial. All subjects were requested to abstain from caffeine on each study day and from any drug, including supplemental vitamins, for the duration of the study.

After admission into the study, subjects were randomized to receive double-blind placebo or 50 mg of sildenafil (Viagra; Pfizer). Two hours later, subjects returned to the laboratory, and standing blood pressure measurements were obtained. At this point, radial artery flow-mediated dilatation (FMD) was measured as described in detail below. Subsequently, a pneumatic cuff placed at the level of the brachial artery and inflated to 200 mm Hg for 15 minutes was used to induce radial artery ischemia. At the end of this period of ischemia, 15 minutes of reperfusion was allowed before FMD was measured again.

Seven days later, subjects returned to the laboratory to receive the alternate study medication (ie, sildenafil or placebo), and the protocol described above was repeated. At the end of the study, 5 subjects had received placebo as the first treatment and the other 5 had received sildenafil. We elected not to test endothelium-independent vasodilators because previous studies have already demonstrated that this cycle of IR induces a specific impairment in endothelium-dependent responses,[3] leaving smooth muscle responsiveness unaltered and, in the case of nitroglycerin, for the risk of positive interaction with sildenafil.

Protocol 2: Effect of Glibenclamide

In a separate protocol, 7 healthy volunteers were administered 5 mg glibenclamide (glyburide; Euglucon, Roche Pharma) 1 hour before the administration of 50 mg sildenafil. This dose has previously been shown to be able to completely inhibit forearm K_{ATP} channels.[17] On glibenclamide administration, a 10% dextrose infusion was started and titrated to maintain blood sugar levels between 80 and 120 mg/dL throughout the study period. Two hours later, these subjects underwent FMD measurement before and after IR as described above. Because of safety concerns related to the potent hypoglycemic effect of glibenclamide requiring continuous adjustment of a dextrose infusion, this protocol was not double-blinded. Importantly, for this protocol, all radial artery analysis was performed in a randomized, investigator-blinded fashion. All subjects except for one also participated in protocol 1. Previous studies have shown that glibenclamide has no independent effect on IR-induced endothelial dysfunction.[16]

Measurement of Radial Artery Diameter and FMD

Subjects were asked to rest for ≥10 minutes in the supine position before measurements were started. Radial artery images were taken with an Advanced Technology Laboratories 3500 instrument with a 7- to 12-MHz linear array transducer. To avoid any bias during image acquisition, a probe holder was used to ensure stable transducer position throughout the studies. End-diastolic, ECG-gated, longitudinal, B-mode images were digitally acquired on a personal computer and stored for off-line analysis. Baseline radial artery diameter was averaged from 6 separate images taken at 5-second intervals. Subsequently, a pneumatic cuff placed at the level of the wrist (ie, distal to the site of radial artery measurement) was inflated to 250 mm Hg for 4 minutes, 30 seconds. FMD was calculated as percent maximum increase of arterial diameter measured at 5-second intervals starting at 30 seconds and ending at 3 minutes, 30 seconds after wrist-cuff deflation. Radial artery diameter was calculated (from trailing edge to leading edge of the intima-blood interface) semiautomatically using a modified version of the Image J software (National Institutes of Health) as well as custom-designed software. Baseline and postischemic (reactive hyperemia) radial blood flows were measured using pulsed-wave Doppler as average velocity-time integral for the first 5 cardiac cycles after cuff deflation multiplied by heart rate and vessel cross-sectional area. The coefficient of variation for brachial artery diameter measurements at rest and during FMD in our laboratory are 3% and 9%, respectively (n=10).

Statistical Analysis

Data are presented as mean ± SEM. Baseline values were compared by use of a paired *t* test (between visits, protocol 1) or an unpaired *t* test (between protocols). The effect of IR on radial artery diameters, reactive hyperemia, and FMD within each study visit (protocols 1 and 2) was tested by use of a paired *t* test. Between-group differences (sildenafil versus placebo) in protocol 1 were analyzed by use of a 2-way ANOVA for repeated measures. Post hoc comparisons were performed with the Bonferroni correction. A value of $P<0.05$ was set as the threshold for significance. StatView version 5 (SAS Institute Inc) was used for statistical analysis.

Results

Radial artery diameter and blood flow measurements are presented in the Table. FMD data are reported in Figures 1 and 2.

Protocol 1

Effect of Sildenafil Administration on Baseline Parameters

Sildenafil administration caused a reduction in mean arterial blood pressure that was not statistically significant (placebo: 93±1 mm Hg; sildenafil: 89±2 mm Hg, P=NS). Sildenafil had no effect on radial artery diameter, blood flow, reactive hyperemia, or FMD before IR (Table and Figure 1, P=NS).

Effect of Ischemia and Reperfusion After Placebo Administration

On the placebo day, radial artery diameter and blood flow returned to baseline values after IR (P=NS for both com-

Radial Artery and Blood Flow Data

	Before IR		After IR	
	Baseline	FMD	Baseline	FMD
Radial artery diameter, mm				
Placebo	2.40±0.1	2.59±0.1	2.37±0.1	2.41±0.1*
Sildenafil	2.38±0.1	2.55±0.1	2.33±0.1	2.47±0.1
Glibenclamide+ sildenafil	2.39±0.1	2.63±0.1	2.41±0.1	2.45±0.1*
Blood flow, mL/min				
Placebo	24±5	164±35	18±3	151±32
Sildenafil	19±5	135±24	17±4	142±24
Glibenclamide+ sildenafil	22±9	115±18	13±6	105±35

Baseline data refer to brachial artery diameter before wrist cuff occlusion. FMD data refer to maximal radial artery diameter in the period between 30 seconds and 3 minutes, 30 seconds after wrist cuff deflation.

*$P<0.01$ vs corresponding value before IR.

pared with before IR). After IR, FMD was significantly blunted compared with pre-IR levels (Table and Figure 1, $P<0.001$ compared with placebo visit, before IR) despite a similar peak hyperemic blood flow (before IR: 807±220%; after IR: 881±203%, P=NS).

Effect of Ischemia and Reperfusion After Sildenafil Administration

On the day that sildenafil was administered, radial artery diameter and blood flow returned to baseline values after IR (P=NS compared with sildenafil before IR and with placebo after IR). Similarly, IR did not affect measures of peak reactive hyperemia (before IR: 971±232%; after IR: 1064±223%, P=NS). Sildenafil administration completely prevented the blunting in FMD that was observed after IR on the placebo day (Figure 1; P=NS compared with sildenafil before IR; $P<0.01$ compared with placebo after IR; 2-way ANOVA).

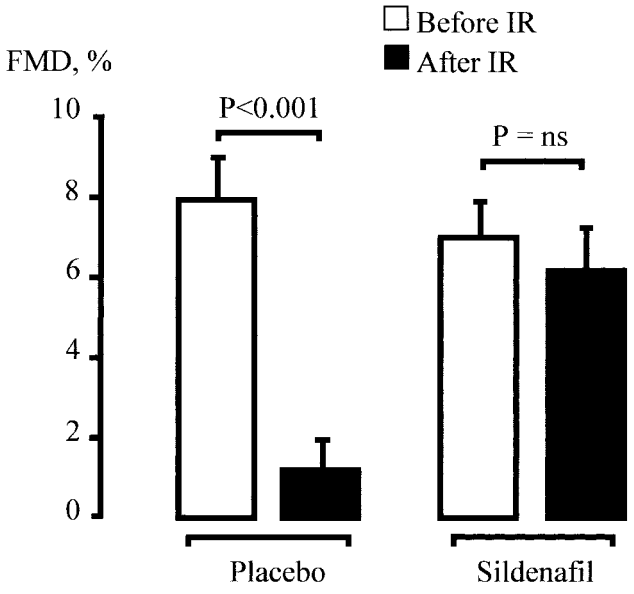

Figure 1. Protocol 1. Percent increase of radial artery diameter during reactive hyperemia before and after IR. Left, On placebo day, FMD was significantly blunted after IR. Right, Sildenafil completely prevented this impairment in endothelium-dependent vasodilation induced by IR. Abbreviations as in Table.

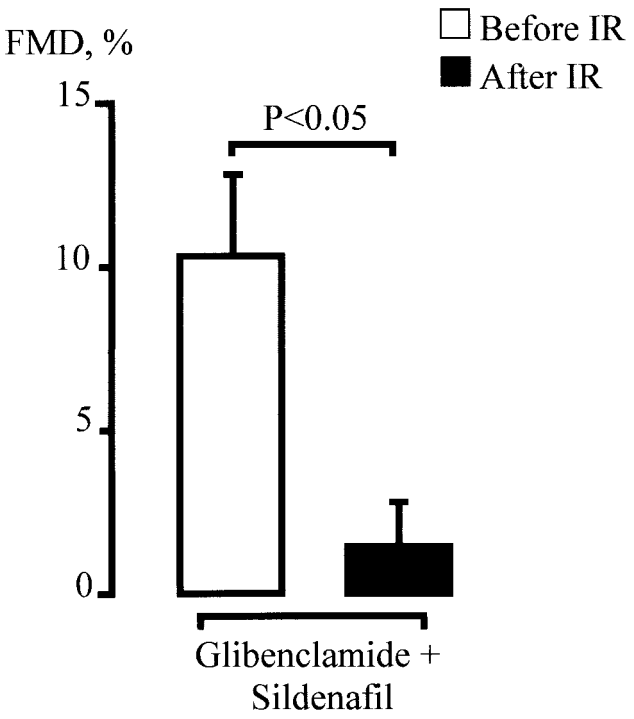

Figure 2. Protocol 2, after administration of glibenclamide and sildenafil. Percent increase of radial artery diameter during reactive hyperemia before and after IR. As for protocol 1 in placebo session, FMD was significantly blunted after IR, demonstrating that glibenclamide inhibits sildenafil-induced endothelial protection against IR. Abbreviations as in Table.

Protocol 2

Effect of Glibenclamide

Radial artery diameter and blood flow data are reported in the Table. Pre-IR baseline radial artery diameter, reactive hyperemia, and FMD were not different after glibenclamide plus sildenafil compared with sildenafil alone. After IR, baseline radial artery diameter returned to the values observed before IR. However, at this time point, despite a similar blood flow stimulus, FMD was blunted to values similar to those observed in protocol 1 on the placebo day, demonstrating a potent inhibitory effect of K_{ATP} channel blockade on sildenafil-induced endothelial protection ($P<0.05$ compared with before IR, Figure 2).

Discussion

In a recent report, Kharbanda et al[3] demonstrated that exposure to IR can impair FMD at the level of forearm conductance vessels while leaving endothelium-independent vasodilation unchanged. In their report, exposure to brief episodes of ischemia, ie, ischemic preconditioning, limited this specific impairment in endothelium-dependent relax-

ation. The data presented here document that these abnormalities caused by IR can also be prevented by pretreatment with sildenafil.

Multiple animal studies have demonstrated that a protective phenotype analogous to ischemic preconditioning can be induced by administration of pharmacological stimuli such as adenosine, bradykinin, nitric oxide donors, and opioids.[4] Importantly, a crucial step in the complex molecular cascades triggered by these mediators, as well as by ischemic preconditioning, appears to be the activation of K_{ATP} channels.[18] Although these biochemical mechanisms were more thoroughly investigated in (cardio)myocytes, K_{ATP} channels have also been identified in endothelial cells,[19] and a recent animal study demonstrated that the blunting in endothelium-dependent dilatation that follows IR can be prevented by exposure to specific agonists of these channels.[16]

Interestingly, multiple lines of evidence have emphasized the role of endogenous (endothelial) and exogenous nitric oxide in the physiology of both ischemic and pharmacological preconditioning[15] via direct and cGMP-mediated opening of K_{ATP} channels.[18,20] This pathway might have clinical relevance, because (1) administration of a nitric oxide donor can reduce evidence of myocardial ischemia during angioplasty[21] and (2) administration of the K_{ATP} channel blockers sulfonylureas, such as glibenclamide, prevents the development of cardiac ischemic preconditioning.[22] Recent research in animal models has reported that sildenafil administration, probably by reducing cGMP catabolism and therefore increasing intracellular bioavailability of this mediator, is associated with a significant reduction of myocardial infarct size and incidence of arrhythmias after coronary artery ligation, an effect that appears to mimic ischemic preconditioning.[10,11,23] Of note, controversial results have also been reported,[12] and, most importantly, the possibility of modulating endothelial sensitivity to IR via changes in cGMP bioavailability has never been tested in humans.

In our study, as previously reported,[24] sildenafil (or the combination of sildenafil and glibenclamide) did not have an independent effect on radial artery diameter, blood flow, or FMD. Importantly, radial artery FMD after IR was significantly blunted on the placebo day, whereas the administration of sildenafil markedly limited this effect. These data demonstrate that although IR is able to induce a significant impairment in endothelium-dependent vasodilation, pretreatment with sildenafil can trigger a molecular cascade leading to a protective phenotype. Suggesting that this effect was mediated by K_{ATP} channel opening, the endothelial protection was completely prevented when a blocker of these channels was administered before sildenafil. Our model does not allow a specific investigation of the molecular mechanisms involved in this protective effect. The exact cellular and subcellular sites of the effects of sildenafil and glibenclamide, as well as the mediators involved, will have to be investigated in in vitro studies. However, because previous studies demonstrate that the IR protocol used here does not affect endothelium-independent vascular responses[3] and because of the evidence that endothelial cells, like smooth muscle cells, possess the apparatus necessary to develop preconditioning,[16] we propose a direct involvement of endothelial cells in our model.

In conclusion, we demonstrate that sildenafil administration can induce potent endothelial protection via opening of K_{ATP} channels. These findings represent the first human evidence of endothelial pharmacological preconditioning and provide a mechanistic explanation for this phenomenon. Further studies are now necessary to investigate in greater detail the mechanism(s) of this effect and, most importantly, its potential clinical implications.

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