Although it is well established that ischemic preconditioning (IPC) and pharmacological preconditioning (PPC) protect cardiac myocytes against reversible and irreversible injury and the genesis of cardiac arrhythmias in all animal species and humans, the effect of IPC and PPC on endothelial dysfunction has been less thoroughly studied. In this regard, the first article to clearly establish that IPC can protect endothelium against injury was published by DeFily and Chilian in 1993. They demonstrated in dogs that IPC preserved the endothelium-mediated coronary dilator responses to 2 endothelial-dependent vasodilators, serotonin and acetylcholine, in the absence of any damage to the smooth muscle cells mediating the dilator response. From a mechanistic standpoint, it has also been known since 1992 that adenosine triphosphate (ATP)-sensitive potassium channels (KATP channels) are an integral part of both acute and delayed IPC against myocardial infarction in animals and man. Subsequently, Katnik and Adams demonstrated that an ATP-sensitive potassium channel was present in rabbit endothelium that was inhibited by the KATP channel antagonist tolnbutamide and glibenclamide. That endothelial KATP channels may be involved as a trigger in IPC to protect isolated guinea pig hearts against endothelial dysfunction was demonstrated recently by Beresewicz et al. These authors showed that the opening of mitochondrial KATP channels by IPC or diazoxide protected the endothelium by reducing the burst of reactive oxygen species, particularly the superoxide anion, which occurs at reperfusion. Thus, strong evidence in animal models indicates the presence of a KATP channel in endothelial cells and suggests that its activation resulting from IPC and PPC results in a protective effect to alleviate endothelial dysfunction after ischemia/reperfusion (I/R) injury.

In spite of these previously published animal studies, which clearly suggest that IPC protects against endothelial dysfunction, the first human study to corroborate these findings was not published until 2001 by Kharbanda et al. These authors studied the effect of forearm I/R on endothelial and neutrophil function in healthy human subjects in which the forearm was made ischemic by inflating a blood pressure cuff to 200 mm Hg for 20 minutes. Before and after I/R, the dilator response to the endothelial-dependent vasodilator acetylcholine and the non–endothelial-dependent dilator nitroglycerin was determined. The upregulation of the neutrophil CD11b in circulating blood also was determined as an index of neutrophil function. IPC was produced by three 5-minute episodes of I/R before the 20-minute ischemic period. I/R in control subjects produced a marked reduction in radial artery dilation to acetylcholine but not to nitroglycerin and an upregulation of CD11b. These responses to I/R were prevented by IPC. Although the mechanism(s) responsible for the protection exerted by IPC were not studied, this observation suggests that clinically there may be ways of pharmacologically preconditioning the vasculature to better increase reflow in patients with vascular injury after surgical procedures.

More recently, the same group of investigators studied the role of ATP-sensitive potassium channels in mediating the effect of IPC in healthy human volunteers subjected to I/R of the forearm. Endothelial function was again studied by assessing the vasodilator responses to intra-arterial injections of acetylcholine before and at 15 minutes after 20 minutes of forearm I/R. In these studies, the authors determined the effect of IPC on acetylcholine-induced vasodilation in the absence and presence of pretreatment with the KATP channel antagonist glibenclamide and by pretreating another group with the KATP channel opener diazoxide alone or in the presence of glibenclamide. IPC prevented endothelial dysfunction produced by I/R as expected, and this protection was prevented by concomitant administration of glibenclamide and IPC. In addition, diazoxide pretreatment mimicked the effect of IPC to prevent endothelial dysfunction, and this effect was blocked by glibenclamide. Importantly, diazoxide did not affect the vasodilator responses to acetylcholine in the absence of I/R. These data suggest that KATP channels play a central role in I/R-induced endothelial dysfunction in humans and that modulating channel function by openers of KATP channels may have therapeutic benefit in treating patients with coronary vascular or peripheral vascular disease. That diazoxide mimicked the effect of IPC also suggests that the mitochondrial KATP channel may be involved in producing this beneficial effect because diazoxide is a putative selective mito-KATP channel agonist. Similar findings were initially reported by Tomai and colleagues in humans undergoing repeated episodes of angioplasty; they showed that glibenclamide blocked the beneficial effect of IPC in cardiomyocytes. These studies suggest that activation of myocardial or endothelial localized KATP channels is beneficial in patients with ischemic heart disease and reduces injury to both cardiac myocytes and coronary endothelial cells.

Interest has been increasing in cyclic guanosine monophosphate (cGMP)–dependent phosphodiesterase-5 (PDE-5) inhibitors for their potential beneficial effects on the vasculature and the ischemic reperfused myocardium (Figure). These agents, which include sildenafil, vardenafil, and tadalafil, were developed for their potent effects on the corpus cavernosum and for treating men with erectile dysfunction (ED). The enzyme that they antagonize, PDE-5, is found in high abundance in most vascular beds and it has recently been found in cardiac canine myocytes. This enzyme prevents the breakdown of nitric oxide (NO)–driven cGMP, primarily in vascular smooth muscle cells.
Sildenafil Citrate (VIAGRA) 

Vasodilation

Adenosine/ Bradykinin /Acetylcholine

+ + + Kinases ?

eNOS

NO

Guanylyl Cyclase

GTP

cGMP

PDE-5

Inhibition(-)

Opening of $K_{\text{ATP}}$ Channel

CARDIOPROTECTION

Kukreja et al. (11)

Proposed mechanism of cardioprotection produced by sildenafil. The vasodilatory action of sildenafil might release endogenous mediators of preconditioning, such as adenosine and bradykinin, that may trigger a signaling pathway in cardiac myocytes, resulting in the release of nitric oxide (NO). An increase in NO could activate guanylyl cyclase (GC) and form cyclic GMP. Cyclic GMP would then activate PKG, which has been shown to activate mitochondrial KATP–linked mechanism. These potential signaling pathways are in basic agreement with the results of Han et al., who recently showed that an NO–cGMP–PKG pathway contributes to the phosphorylation and activation of mitochondrial $K_{\text{ATP}}$ channels in isolated rabbit ventricular myocytes. Further support for a cardioprotective role of sildenafil came in a study by Nagy et al., who reported that sildenafil reduced the severity of ventricular arrhythmias in dogs 24 hours after oral administration. In contrast, Swissa et al. observed that acutely administered sildenafil in combination with an NO donor, nitroprusside or nitroglycerin, was proarrhythmic in nonischemic buffer-perfused isolated right ventricular walls of swine hearts. Reffelman and Kloner found that sildenafil produced no cardioprotective effect to reduce infarct size and to maintain microvascular function in intact rabbit hearts in spite of a drug-induced reduction in preload and afterload, 2 major determinants of myocardial oxygen consumption. The reasons for the discrepancies observed in the latter 2 studies is not known, but they may be the result of protocol differences, a drug interaction in the study in which sildenafil was given in combination with an NO donor, or, possibly, species differences between dogs and pigs. In the rabbit study, the result of differences in dose used may have been critical, but other undetermined factors also may be involved. Nevertheless, the majority of evidence to date strongly supports the notion that sildenafil is markedly cardioprotective in most animal species studied.

A novel landmark study published by Ockaili et al. found that sildenafil produces a marked preconditioning-like effect in intact anesthetized rabbit hearts. This effect was observed after both acute treatment with sildenafil given 30 minutes before ischemia and chronic treatment given 24 hours before the index ischemic period. All rabbits were subjected to 30 minutes of regional ischemia and 3 hours of reperfusion after vehicle or drug administration. Infarct size was determined with the triphenyltetrazolium histochemical staining technique. Acute treatment with sildenafil reduced infarct size by 68% and delayed treatment by 41%, respectively. Interestingly, these protective effects were completely blocked by 5-HD, a mitochondrial selective $K_{\text{ATP}}$ channel antagonist, when it was administered 10 minutes before the index ischemic period in either the acute or chronic protocol. In addition, both acute and delayed cardioprotection was observed when sildenafil was administered orally. In 2 more recent publications, Das et al. found that protein kinase C (PKC) was involved in the acute cardioprotective effect of sildenafil to reduce infarct size in rabbits and that this effect may be the result of either PKC-α, PKC-θ, or PKC-δ. Salloum et al. showed that an upregulation of both endothelial and inducible NO synthase occurred in a delayed preconditioning model in mouse hearts subjected to global ischemia 24 hours after sildenafil administration. The selective inducible NO synthase inhibitor 1400W completely abolished sildenafil-induced cardioprotection when administered 30 minutes before index ischemia and 24 hours after sildenafil administration. Taken together, these results suggest that both acute and chronic administration of sildenafil produces a potent cardioprotective effect to reduce infarct size in rabbits and mice via a PKC-, NO-, and mitochondrial $K_{\text{ATP}}$-linked mechanism.
by guest on April 13, 2017

Glibenclamide, a nonselective K<sub>ATP</sub> channel antagonist, was administered at 5 mg 1 hour before administration of sildenafil in a randomized investigator-blinded protocol. Ischemia and reperfusion resulted in a decrease in endothelial function in placebo-treated controls, and this decrease was prevented by prior administration of sildenafil. The protective effect of sildenafil was completely blocked by glibenclamide at a dose that had no effect on endothelial function in the absence of sildenafil. Although the mechanism by which sildenafil produced this beneficial effect at a cellular or molecular level was not studied, this is the first complete study in humans to suggest that sildenafil protects the endothelium from the effects of I/R and that this effect involves the activation of vascular K<sub>ATP</sub> channels. The ability of sildenafil to protect or enhance endothelial function in humans has been suggested by 2 previous studies.22,23 Katz et al<sup>22</sup> showed that sildenafil administered acutely was protective in both studies. Halcox et al<sup>23</sup> also found that 100 mg of sildenafil dilated epicardial coronary arteries, improved endothelial dysfunction in the brachial arterial branch, and inhibited platelet activation in patients with coronary artery disease. In this study, sildenafil also reduced exercise-induced ischemia and had an intermediate effect when compared with placebo and isosorbide dinitrate. It is interesting to note that sildenafil was effective in both normal healthy volunteers and patients with chronic heart failure or coronary artery disease because few studies have been done in humans comparing the effects of cardioprotective drugs in normal and diseased hearts; different responses may be expected on the basis of animal studies.<sup>1</sup>

In conclusion, the present results obtained in humans and previous studies in animals suggest that cGMP and PDE-5 inhibitors may have important effects separate from their major expected on the basis of animal studies.1 That PDE-5 inhibitors act via vascular and myocardial preconditioning phenomenon pharmacologically. Future experimental studies are needed to elucidate the cellular mechanisms by which PDE-5 inhibition and K<sub>ATP</sub> channels protect endothelial function from I/R injury.

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