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COMMENTARY

Cardiovascular protection with sildenafil following chronic inhibition of nitric oxide synthase

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During the past 18 years, sildenafil has evolved from a potential anti-angina drug to an on-demand treatment for erectile dysfunction and more recently to a new orally active treatment for pulmonary hypertension. Recent studies suggest that the drug has powerful cardioprotective effect against ischemia/reperfusion injury, doxorubicin-induced cardiomyopathy and anti-hypertensive effect induced by chronic inhibition of nitric oxide synthase in animals. Based on several recent basic and clinical studies, it is clear that sildenafil and other clinically approved type-5 phosphodiesterase-5 inhibitors including vardenafil and tadalafil will eventually be developed for several cardiovascular indications including essential hypertension, endothelial dysfunction, ischemia/reperfusion injury, myocardial infarction, ventricular remodeling and heart failure.
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Abbreviations: cAMP, cyclic adenosine monophosphate; cGMP, cyclic gunosine monophosphate; ED, erectile dysfunction; eNOS, endothelial nitric oxide synthase; L-NAME, N^{ω}-nitro-L-arginine methyl ester; NO, nitric oxide; NOS, nitric oxide synthase; PDE-5, phosphodiesterase-5; PKG, protein kinase G

Phosphodiesterase type-5 (PDE-5) inhibitors are a class of vasoactive drugs that have been developed for treatment of erectile dysfunction (ED) (Boolell *et al*., 1996; Porst *et al*., 2001). These compounds antagonize, PDE-5, which is found in most vascular beds as well as cardiac myocytes (Senzaki *et al*., 2001; Das *et al*., 2005). PDE-5 inhibitors prevent the breakdown of nitric oxide (NO)-driven cyclic guanosine monophosphate (cGMP), primarily in vascular smooth muscle cells, and therefore are potent vasodilators. PDE-5 enzyme is found in high abundance in the corpus cavernosum and in pulmonary artery smooth muscle, where its inhibition produces an increase in penile blood flow and a reduction in pulmonary vascular resistance, respectively (Sebkhi *et al*., 2003). Corbin and Francis (1999) showed that the regulatory domain in the amino-terminal portion of PDE-5 contains a phosphorylation site (Ser 92), two allosteric cGMP-binding sites and at least a portion of the dimerization domain. The catalytic domain in the carboxy-terminal portion of the protein contains the two Zn^{2+}-binding motifs and a cGMP substrate-binding site. Most known PDE-5 inhibitors compete with the substrate cGMP for binding to the protein at the catalytic site. Although cGMP binding to the catalytic site stimulates cyclic-nucleotide binding to the allosteric sites, inhibitors do not elicit the same property, and Ser 92 phosphorylation has no effect on inhibitor binding (Corbin and Francis, 1999). Sildenafil citrate (Viagra) is the first PDE-5 inhibitor approved for treatment of ED. Two additional agents in this class tadalafil (Cialis) and vardenafil (Levitra) are also in clinical use for management of ED. Vardenafil has been shown in a rabbit model to increase intracavernosal pressure more quickly and to a greater extent than sildenafil (Saenz de Tejada *et al*., 2001). Vardenafil has a similar duration of action to sildenafil, but is more potent and selective biochemically. It has also been shown in clinical trials to have a high efficacy and low adverse-event profile in a population with mixed ED aetiologies (Porst *et al*., 2001). Tadalafil is a long-acting PDE-5 inhibitor which is effective for up to 36 h in the majority of men (Eardley and Cartledge, 2002). Except for the marked hypotensive effect of these drugs in men when combined with nitrates and α-blockers used to treat essential hypertension, a large body of evidence has shown that PDE-5 inhibitors are generally safe, being used by millions of patients around the world.

In recent years, there has been considerable interest in studying the effect of sildenafil and other PDE-5 inhibitors in protection against ischaemia/reperfusion injury (reviewed in Kukreja *et al*., 2004, 2005). We hypothesized that the vasodilator action of PDE-5 inhibitors could potentially

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release endogenous mediators of cardioprotection, including adenosine and bradykinin from endothelial cells, that may trigger a signalling cascade (through the action of kinases including protein kinase C and other mitogen-activated protein kinases) and generation of NO by phosphorylation of endothelial NO synthase (eNOS). NO activates guanlylate cyclase resulting in enhanced formation of cGMP, which activates protein kinase G (PKG). PKG can subsequently open mitochondrial K_{ATP} channels resulting in cardioprotective effects against ischaemia/reperfusion injury (Kukreja *et al.*, 2004, 2005; Das *et al.*, 2006). We initially showed that rabbits treated with sildenafil or vardenafil had reduced infarct size after ischaemia/reperfusion (Ockaili *et al.*, 2002; Salloum *et al.*, 2006). Recently, Sesti *et al.* (2006) also confirmed these results with tadalafil. The protective effect of sildenafil has been duplicated in several other experimental models including infant rabbits (Bremer *et al.*, 2005), the mouse isolated perfused heart (Salloum *et al.*, 2003), adult cardiac myocytes subjected to simulated ischaemia and reoxygenation *in vitro* (Das *et al.*, 2005) and doxorubicin-induced cardiomyopathy in mice (Fisher *et al.*, 2005). Sildenafil preconditioning has also been shown to reduce serious ventricular arrhythmias during ischaemia in dogs (Nagy *et al.*, 2004) . Very recently, we demonstrated that administration of sildenafil or vardenafil at reperfusion after lethal ischaemia reduced infarct size by opening the mitochondrial K_{ATP} channel, similar to the preconditioning-like effect (Salloum *et al.*, 2006). In contrast, the antianginal drug, nitroglycerine failed to exhibit similar infarct-limiting effects, suggesting that the protection at reperfusion was selectively due to inhibition of PDE-5. In general, there is overwhelming evidence for the cardioprotective effect of PDE-5 inhibitors against ischaemia/reperfusion injury.

PDE-5 inhibitors are also highly effective vasodilators in the systemic circulation. A recent clinical study suggested that PDE-5 inhibitors might be a new class of drug that can potentially be used for the treatment of essential hypertension (Oliver *et al.*, 2006). Active treatment with sildenafil significantly reduced both ambulatory and clinical blood pressure by an extent similar to that observed with the other classes of antihypertensive drugs. PDE-5 inhibitors may also be effective in the treatment of patients with heart failure because of their effects on preload and afterload. Endothelial function is severely limited in chronic congestive heart failure and sildenafil has been shown to improve endothelial function in such patients (Katz *et al.*, 2000).

An important property of sildenafil is its ability to increase eNOS and inducible NO synthase proteins in the heart (Salloum *et al.*, 2003) and cardiomyocytes (Das *et al.*, 2005), and this has a direct cause and effect relationship in protection against myocardial infarction (Salloum *et al.*, 2003) and simulated ischaemia/reoxygenation-induced necrosis, as well as apoptosis in cardiomyocytes (Das *et al.*, 2005). Because of the ability of sildenafil to augment NO synthase (NOS) and cGMP levels, it is logical to hypothesize that cardiovascular dysfunction induced by chronic NOS inhibition would be alleviated by concomitant treatment with sildenafil. In this issue of the *British Journal of Pharmacology*, Rossoni *et al.* (2007) describe the beneficial

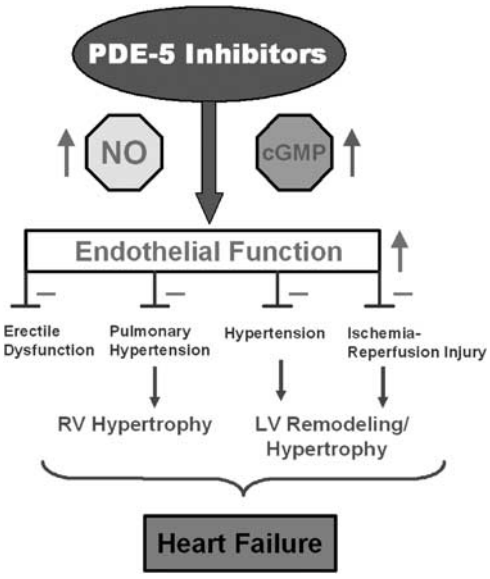

Figure 1 Cardiovascular protection with PDE-5 inhibitors. See text for description. Abbreviations: RV, right ventricle; LV, left ventricle.

effect of chronic sildenafil therapy on cardiovascular alterations induced by NOS inhibition with a non-selective NOS inhibitor, N^{ω}-nitro-L-arginine methyl ester (L-NAME). Sildenafil was given orally at doses of 0.37, 0.75 or 1.5 mg kg^{-1} day^{-1} for 4 weeks, either alone or with L-NAME (35–40 mg kg^{-1} day^{-1}) in the drinking water. The results show that sildenafil dose-dependently prevented the increase of systolic blood pressure, improved post-ischaemic functional recovery and reduced the release of creatine kinase and lactate dehydrogenase in the L-NAME-treated rats. Moreover, sildenafil restored the drop in myocardial cGMP and cAMP, the urinary levels of NO and attenuated the excretion of the vasoconstrictor metabolites of arachidonic acid including thromboxane B_2, 6-keto-$PGF_{1\alpha}$ and 8-iso-$PGF_{2\alpha}$ in L-NAME-treated rats. These results support the concept that sildenafil and possibly other PDE-5 inhibitors have antihypertensive as well as cardioprotective effects against ischaemia/reperfusion injury. The authors further showed that sildenafil therapy restored the vasorelaxant effect of acetylcholine in L-NAME-treated rats, which is in line with previous studies showing improvement of endothelium-dependent vasodilatation with sildenafil (Katz *et al.*, 2000; Gori *et al.*, 2005). In general, these data are very interesting because the authors were the first to provide evidence for the beneficial effect of sildenafil against cardiovascular injury in an experimental setting where NOS was chronically inhibited.

Since 1989, sildenafil has evolved from a potential antiangina drug to an on-demand oral treatment for ED and more recently to a new orally active treatment for pulmonary hypertension (Revatio) (Ghofrani *et al.*, 2006). Based on several clinical and animal studies including those of Rossoni *et al.* (2007), it is clear that sildenafil and other PDE-5 inhibitors will eventually be developed for a number of cardiovascular indications including essential hypertension, endothelial dysfunction, ischaemia/reperfusion injury,

myocardial infarction, ventricular remodelling and heart failure as outlined in Figure 1.

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