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PDE5 inhibitors as therapeutics for heart disease, diabetes and cancer

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Abstract

The phosphodiesterase 5 (PDE5) inhibitors, including sildenafil (Viagra™), vardenafil (Levitra™), and tadalafil (Cialis™) have been developed for treatment of erectile dysfunction. Moreover, sildenafil and tadalafil are used for the management of pulmonary arterial hypertension in patients. Since our first report showing the cardioprotective effect of sildenafil in 2002, there has been tremendous growth of preclinical and clinical studies on the use of PDE5 inhibitors for cardiovascular diseases and cancer. Numerous animal studies have demonstrated that PDE5 inhibitors have powerful protective effect against myocardial ischemia/reperfusion (I/R) injury, doxorubicin cardiotoxicity, ischemic and diabetic cardiomyopathy, cardiac hypertrophy, Duchenne muscular dystrophy and the improvement stem cell efficacy for myocardial repair. Mechanistically, PDE5 inhibitors protect the heart against I/R injury through increased expression of nitric oxide synthases, activation of protein kinase G (PKG), PKG-dependent hydrogen sulfide generation, and phosphorylation of glycogen synthase kinase-3β - a master switch immediately proximal to mitochondrial permeability transition pore and the end effector of cardioprotection. In addition, PDE5 inhibitors enhance the sensitivity of certain types of cancer to standard chemotherapeutic drugs, including doxorubicin. Many clinical trials with PDE5 inhibitors have focused on the potential cardiovascular and cancer benefits. Despite mixed results of these clinical trials, there is continuing strong interest by basic scientists and clinical investigators in exploring their new clinical uses. It is our hope that future new mechanistic investigations and carefully designed clinical trials would help in reaping additional benefits of PDE5 inhibitors for cardiovascular disease and cancer in patients.

1. Introduction

Phosphodiesterases (PDEs) are metallohydrolases that catalyze the breakdown of cAMP or cGMP into the inactive 5′-AMP or GMP, thus modulating the duration and intensity of their intracellular response. PDEs are found in all tissues, but their distribution and type varies

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Conflict of interest

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among different tissues (Wallis et al., 1999). PDEs have 11 families (PDE1–PDE11) that are encoded by 21 different genes. More than 80 enzyme variants are generated from multiple promoters and as a consequence of alternative splicing (Bender & Beavo, 2006). PDE1 through PDE3, PDE10, and PDE11 are dual-specificity esterases because they hydrolyze both cAMP and cGMP; PDE4, PDE7, and PDE8 specifically degrade cAMP; and PDE5, PDE6, and PDE9 hydrolyze cGMP (Rotella, 2002). The NH2-terminal portion of the PDE enzyme may undergo phosphorylation/dephosphorylation events, binding of Ca2+/calmodulin, and allosteric binding of cGMP and can mediate interactions with other proteins. PDE1, PDE3, PDE4, and PDE5 contain phosphorylation sites for various kinases. PDE1 also contains Ca2+-calmodulin binding sites, and stimuli that increase or decrease intracellular Ca2+ thereby profoundly affecting its activity. PDE2, PDE5, PDE6, PDE10 and PDE11 contain allosteric binding sites for cGMP called GAF (cGMP-binding PDEs, Anabaena Adenylyl cyclase, and Escherichia coli FhlA) domains (Maurice et al., 2014). The binding of cGMP to GAFB in PDE2 activates the enzyme, whereas the binding of cGMP to GAFA in PDE5 favors protein kinase G (PKG)-mediated phosphorylation and activation of the enzyme (Francis et al., 2011; Kukreja et al., 2012). Phosphorylation of PDE5 by PKG serves to increase its cGMP affinity and represents an alternative mode of regulatory feedback inhibition within the cGMP/PKG signaling cascade, thus normalizing levels of cGMP (Lucas et al., 2000). PKG activation phosphorylates numerous intracellular proteins that in turn regulate many primary physiological functions such as modulation of vascular tone, vasorelaxation in vascular smooth muscle, endothelial permeability, and cell differentiation and proliferation (Hofmann et al., 2000). The PDEs can serve in different functional compartments in cells because they are not co-localized with each other. They are also distributed inside the cell at critical sites and thus regulate local cAMP or cGMP dynamics in space and time. Such compartmentalization of signaling components allows the extracellular signal to propagate inside the cell along defined and specific pathways within the network.

In recent years, there has been tremendous interest in identifying new clinical uses of PDE5 inhibitors in various ailments including cardiovascular disease (Kukreja et al., 2004; Kukreja et al., 2005; Kukreja et al., 2011), diabetes (Giannetta et al., 2012; Koka et al., 2012; Koka et al., 2013; Koka et al., 2014; Varma et al., 2012) and cancer (Black et al., 2008; Booth et al., 2014a; Booth et al., 2014c; Booth et al., 2014b; Das et al., 2010; Hamilton et al., 2013; Resnick et al., 2009; Roberts et al., 2014). Today, close to 100 clinical trials (http://www.clinicaltrials.gov) with PDE5 inhibitors focusing on the potential cardiovascular benefits have been completed or are ongoing.

2. PDE5 Inhibitors in Cardioprotection

2.1. PDE5 expression in heart

There has been a dominant view that PDE5 is not present in the myocardium mainly based on the earlier studies by Ito et al (Ito et al., 1996) and Wallis et al. (Wallis et al., 1999), who failed to detect enzyme activity and/or immunoreactive bands of PDE5 in human ventricular tissues. Immunohistochemical studies have demonstrated the presence of PDE5 in vascular and bronchial smooth muscle and in platelets. Moreover, there is evidence for PDE5

expression in canine (Senzaki et al., 2001) and mouse heart (Das et al., 2005). In contrast to previous studies in human heart samples, Loughney et al. (Loughney et al., 1998) detected PDE5 mRNA in human heart tissue. Importantly, PDE5 is up-regulated 2- to 6-fold in experimental mice and human heart disease (Nagendran et al., 2007; Pokreisz et al., 2009; Takimoto et al., 2005; Vandeput et al., 2009), potentially increasing its influence and pharmacologic impact from its subsequent inhibition. Gene-silencing studies further supported the specificity and selectivity of these results (Zhang et al., 2008). PDE5 contributed approximately 22 and 43% of the cytosolic cGMP-hydrolytic activity in preparations from normal and failing mouse hearts, respectively (Vandeput et al., 2009). Despite these multiple reports on the expression of PDE5 in heart, Hoffmann and colleagues (Lukowski et al., 2014) expressed concerns that the methods used in these studies did not distinguish which cell types in the heart were actually contributing to the PDE5 signal.

2.2. PDE5 inhibitors in protection against ischemia/reperfusion injury

Our landmark study published in 2002 demonstrated the protective effect of sildenafil against experimental myocardial ischemia/reperfusion (I/R) injury in rabbits (Ockaili et al., 2002). In this investigation, rabbits treated with sildenafil (0.7 mg/kg, i.v.) prior to index regional ischemia significantly reduced infarct size. The protective effect of sildenafil as well as vardenafil was observed when the drug was given at the time of reperfusion (Salloum et al., 2007b). Even though sildenafil caused significant decline in blood pressure when administered i.v. prior to I/R (Ockaili et al., 2002), a much milder effect on hemodynamics was observed when these drugs were given in a slow infusion protocol in these studies. Interestingly, the cardioprotective effects of PDE5 inhibitors were not exclusive to adult rabbits. We showed that sildenafil reduced infarct size and preserved left ventricular cardiac output and aortic velocity time integral following I/R in infant rabbits as well (Bremer et al., 2005).

Similar to cardiac preconditioning (Schultz et al., 1997), cardioprotection with sildenafil and vardenafil at reperfusion was mediated by opening of mitochondrial K_{ATP} (mitoK_{ATP}) because 5-hydroxydecaoate blocked this protection as indicated by a significant increase in infarct size (Salloum et al., 2007b). Opening of mitoK_{ATP} channels causes partial compensation of the membrane potential, which enables additional protons to be pumped out to form a H^+ electrochemical gradient for both ATP synthesis and Ca^{2+} transport (Szewczyk et al., 1993). In addition to opening of mitoKATP channel, we have shown that sildenafil requires mitochondrial Ca^{2+}-activated K (mitoK_{Ca}) channels since the blocker of this channel, paxilline blunted the infarct-sparing effects of sildenafil (Wang et al., 2008). Other studies have shown that the cGMP/PKG pathway also confers ischemic post-conditioning protection in part by delaying normalization of pH during reperfusion, probably via PKG-dependent inhibition of Na^+/H^+-exchanger in rat heart (Inserte et al., 2011).

2.3. Protection of adult cardiomyocytes against ischemic injury

To examine whether the cardioprotective effect of sildenafil was independent of the vasculature and systemic hemodynamics, we studied its effect in protection of adult cardiomyocytes against simulated ischemia/reoxygenation injury (Das et al., 2005). In these

studies, the isolated adult murine cardiomyocytes were subjected to in vitro simulated ischemia for 40 minutes by replacing the cell medium with an “ischemia buffer”. Treatment with sildenafil significantly reduced necrosis and apoptosis in cardiomyocytes treated with sildenafil. These findings illustrated that the cardioprotective effects of sildenafil in vivo cannot be solely attributable to its vasoactive properties.

2.4. Protection against ischemic cardiomyopathy

Sildenafil or tadalafil treatment immediately after myocardial infarction attenuated ischemic cardiomyopathy as indicated by improvement in cardiac function, improved survival rate and reduction in apoptosis in the border zone of the infarcted myocardium (Salloum FN, 2014; Salloum et al., 2008). Moreover, sildenafil treatment beginning at three days post-MI also reduced the progression of heart failure, suggesting that PDE5 inhibition can have beneficial effect in patients with advanced heart failure (Chau et al., 2011). In these studies, PKG activation with sildenafil was associated with the inhibition of Rho kinase which is known to suppress left ventricular remodeling following MI in mice (Noma et al., 2006).

2.5. Improving therapeutic potential of stem cells for treatment of heart failure

Although cardiac performance by cell-based therapy has improved, unsatisfactory cell retention and transplant survival still plague this technique. The current transplantation strategies achieve modest engraftment of donor stem cells in the infarcted myocardium, primarily due to the rapid and massive loss of donor stem cells (Muller-Ehmsen et al., 2002; Pagani et al., 2003). Enhancing stem cell survival in the ischemic microenvironment is of paramount importance in improving cardiac regeneration. We recently reported the feasibility of PDE5 inhibition strategy to precondition human adipose stem cells (ASCs) for improving their efficacy in vivo after transplantation in the post-ischemic heart (Hoke et al., 2012). Preconditioning of ASCs with sildenafil or targeted PDE5 gene-silencing approach significantly improved their ability to survive ischemia/reoxygenation injury in vitro. The preconditioned ASCs showed significant release of pro-angiogenic/pro-survival growth factors including VEGF, b-FGF, IGF and Ang-1. The intramyocardial injection of preconditioned ASCs into the border zone following myocardial infarction induced angiogenesis, suppressed fibrosis, and decreased apoptosis and significantly improved cardiac function. These studies suggest that in vitro preconditioning with PDE5 inhibition can be a useful approach to improve stem cell therapy for treatment of ischemic cardiomyopathy in patients.

2.6. Protection against cardiac hypertrophy

Chronic administration of sildenafil prevented and reversed cardiac hypertrophy induced by transverse aortic constriction (Takimoto et al., 2005). In these studies, sildenafil treatment suppressed chronic pressure overload-induced chamber as well as myocyte hypertrophy and improved heart function. A recent study has also shown that chronic treatment with sildenafil attenuated LV remodeling and exercise intolerance following chronic mitral regurgitation (Kim et al., 2012). This benefit was suggested to be associated with the anti-apoptotic, anti-inflammatory effects of sildenafil. Sildenafil also reversed pre-established hypertrophy induced by pressure overload while restoring chamber function to normal. PDE5 increased in pressure-loaded hearts which was associated with increased cGMP

catabolism. PDE5 inhibition led to restoration of cGMP signaling and activation of PKG. The anti-hypertrophic effects coincided with activation of PKG, and its targets included regulator of G protein–coupled signaling-2, as well as calcineurin-NFAT and transient receptor potential channel 6, one of the nonselective and non–voltage-gated ion channels that convey signaling information linked to a broad range of sensory inputs (Zhang & Kass, 2011). In contrast, the antihypertrophic role of PKG has been questioned recently because its deletion in cardiomyocytes did not affect the development of hypertrophy induced by transaortic constriction or long-term infusion of isoproterenol in mice (Lukowski et al., 2010).

More recently, it was shown that the cardioprotective effect of sildenafil in female mice is dependent upon estrogen through a mechanism involving cardiomyocyte eNOS-dependent cGMP synthesis and PKGIα (Sasaki et al., 2014). This study showed that ovariectomy prior to pressure overload abolished the anti-hypertrophic effects of sildenafil, which was restored upon estrogen replacement. Interestingly, modulation of the eNOS/cGMP/PKG axis with sildenafil was completely independent of estrogen in male hearts suggesting the estrogen-dependence of this pathway in females.

2.7. Prevention of doxorubicin-induced cardiomyopathy

Doxorubicin (DOX) is one of the most powerful and widely used anti-cancer drugs in clinics. In particular, the cumulative doses over 550 mg/m^2 increase the risk of developing cardiac side effects, including congestive heart failure (CHF) and dilated cardiomyopathy (Singal & Iliskovic, 1998). The heart failure caused by doxorubicin is characterized by damage resulting from the disintegration of the myofibrillar array, mitochondrial injury, and cardiomyocyte apoptosis, leading to the loss of functional myocardium. Reduction in fractional shortening and abnormalities in the nonspecific T wave and ST-T segment of EKG are typically observed in DOX-induced ventricular dysfunction (Friess et al., 1985). Treatment with sildenafil prior to doxorubicin inhibited cardiomyocyte apoptosis, preserved mitochondrial membrane potential ($\Delta\psi_m$), myofibrillar integrity and prevented LV dysfunction as well as ST segment prolongation (Fisher et al., 2005). Similarly, tadalafil, the long-acting PDE5 inhibitor improved LV function and prevented cardiomyocyte apoptosis in doxorubicin-induced cardiomyopathy through mechanisms involving up-regulation of cGMP, PKG activity, and MnSOD level without interfering with the chemotherapeutic benefits of doxorubicin (Koka et al., 2010).

2.8. Duchenne muscular dystrophy

Duchenne muscular dystrophy (DMD) is a progressive and fatal genetic disorder of muscle degeneration. Patients with DMD lack dystrophin as a result of mutations in the X-linked dystrophin gene. The loss of dystrophin leads to severe skeletal muscle pathologies and cardiomyopathy, a delayed symptom of the disease that usually develops by the second decade of life, with more than 90% of patients presenting clinical symptoms by 18 y of age (38). Dystrophin-deficient mice (mdx mice) show cardiac dysfunction with decrease in diastolic function followed by systolic dysfunction later in life. Chronic treatment with sildenafil reduced functional deficits in the cardiac performance of aged mdx mice without any effect on normal cardiac function in wild type controls (Adamo et al., 2010). When

sildenafil treatment was started after cardiomyopathy had developed; the established symptoms were rapidly reversed within a few days. Based on these findings, clinical trials were initiated to test the efficiency of PDE5 inhibitors as treatment for DMD (e.g., NCT01168908). However, a randomized study of oral sildenafil for treatment of children with PAH showed a possible dose-related increase in mortality after 2 years of sildenafil treatment (Barst et al., 2012). The US Food and Drug Administration (FDA) issued a recommendation not to prescribe sildenafil to children with PAH. This FDA decision led to suspension of the sildenafil clinical trial on treatment of heart dysfunction in children with muscular dystrophy. Meanwhile, another randomized, double-blind, placebo-controlled trial (ClinicalTrials.gov, number NCT01168908) showed that sildenafil does not improve cardiomyopathy in Duchenne/Becker muscular dystrophy (DBMD). Due to the higher number of subjects worsening on sildenafil, the Data and Safety Monitoring Board recommended early termination of this study. The results of this trial suggest that sildenafil is unlikely to improve cardiac function in adults with DBMD (Leung et al., 2014).

2.9. Role of gasotransmitters in cardioprotective signaling with PDE5 inhibition

Since the discovery of nitric oxide (NO) as a signaling molecule, two other toxic gases – carbon monoxide (CO) and hydrogen sulfide (H2S) have been involved in the physiological and pathophysiological functions of cells. These gases, termed “gasotransmitters”, play an increasingly important role in understanding how signaling into and between cells is modulated and fine-tuned. In fact, a large number of stimuli such as brief episodes of ischemia (Jones et al., 1999), endotoxin-derivatives (Xi et al., 1999a; Xi et al., 1999b; Xi & Kukreja, 2000; Zhao et al., 1997), agonists of G protein-coupled receptors (Tejero-Taldo et al., 2002; Xi et al., 2002; Zhao et al., 2000; Zhao & Kukreja, 2002) induce cardioprotective effect in an iNOS-dependent manner. Additionally, adenosine A1 receptor (A1AR) mediated the cytoprotective effects of sildenafil since it failed to reduce cell death in adult cardiomyocytes isolated from A1AR knockout mice or wild type cardiomyocytes treated with the A1AR antagonist, 8-cyclopentyl-1,3-dipropyxanthine (Salloum et al., 2007a).

We demonstrated that up-regulation of iNOS and eNOS played critical role in cardioprotection with sildenafil. In the intact murine myocardium, protection induced following 24 hrs of treatment with sildenafil was completely abolished with 1400W, a selective inhibitor of iNOS (Salloum et al., 2003). In addition, NOS-dependent mechanism in sildenafil-induced protection played a crucial role in the inhibition of apoptosis and necrosis in cardiomyocytes as well. This was demonstrated by absence of the protective effect of sildenafil in cardiomyocytes derived from iNOS and eNOS gene knock-out mice (Das et al., 2005). Further studies in adult mouse cardiomyocytes and intact heart demonstrated the role of PKG, ERK phosphorylation and glycogen synthase kinase-3β (GSK-3β) as key players promoting protection of these cells against simulated ischemia with sildenafil (Das et al., 2006; Das et al., 2008; Das et al., 2009).

H2S is produced enzymatically on a continuous basis at micromolar levels in mammals in the cardiovascular system. H2S has been shown to protect the heart via opening of KATP channel (Elrod et al., 2007). The H2S-producing enzyme, cystathionine-γ-lyase (CSE), is expressed in the heart and administration of the H2S donor, sodium hydrosulfide, reduced

infarct size after I/R. We demonstrated that protection against I/R with tadalafil was abolished with the PKG inhibitor KT5823 (Salloum et al., 2009). Moreover, the protective effect of tadalafil was blunted by treatment with a CSE inhibitor, dl-propargylglycine (PAG), as well as in CSE-knockout mice suggesting a definite role of endogenous H2S signaling in cardioprotection with tadalafil. Cinaciguat (formerly BAY 58-2667) is a novel NO-independent activator of soluble guanylate cyclase (sGC), which induces cGMP-generation and vasodilation in diseased vessels. Cinaciguat treatment caused significant reduction in infarct size, when given as pretreatment or reperfusion therapy. Interestingly, this drug increased the expression of CSE and augmented H2S levels in the heart (Salloum et al., 2012). Thus cGMP generating drugs could be a potential source of producing therapeutic levels of H2S.

More recently, it has been shown that sildenafil promotes the production of carbon monoxide and nitric oxide, by stimulating the expression of the inducible isoforms of heme oxygenase (HO-1) and iNOS in vascular smooth muscle cells (Liu et al., 2012). Interestingly, sildenafil stimulated the expression of HO-1 and iNOS via the ROS- NF-E2-related factor-2 and soluble guanylate-cGMP pathway. Thus sildenafil and potentially other PDE5 inhibitors may have therapeutic benefit in a variety of pathologies, preventable or treatable by gasotransmitters including NO, H2S or CO.

3. PDE5 inhibitors in Diabetes

3.1. Protection against endothelial dysfunction

As recently reviewed by Kloner and colleagues (Kloner et al., 2011), in addition to the well-defined vascular protective effects of PDE5 inhibitors in non-diabetic conditions (Aversa et al., 2007; Mazo et al., 2006; Rosano et al., 2005), several pre-clinical and clinical studies have also focused on the effects of PDE5 inhibitors on arterial endothelium, which is adversely affected by hyperglycemia in type 2 diabetes (T2D). Based on a possible link between painful neuropathy and insufficient NO synthesis under diabetic conditions, PDE5 inhibitors have been tested for prevention of diabetic neuropathy and vasculopathy. For example, administration of sildenafil to the diabetic animals led to increased sensitivity to painful stimuli, but the pain threshold was raised (Patil et al., 2004). In a double-blind placebo-controlled trial in 16 patients with T2D, flow-mediated dilatation (FMD) - a measure of NO-mediated endothelial function-improved significantly with both acute and chronic daily administration of 25 mg sildenafil (Desouza et al., 2002). Similarly, another study in 20 T2D patients also showed significant improvement in endothelial function following both acute (100 mg/day for 3 days) and chronic (25 mg t.i.d. for 4 weeks) treatment with sildenafil (Aversa et al., 2008), although one placebo-controlled cross-over study with single-dose of 100 mg sildenafil in 40 patients with T2D showed no significant change in FMD (Stirban et al., 2009). Interestingly, endothelial functional improvement following daily treatment with 50 mg sildenafil for 30 days in 40 patients with T2D was also associated with a significantly reduced albuminuria and glycated hemoglobin (HbA1c) (Grover-Paez et al., 2007), possibly through sildenafil-induced improvement in pancreatic endothelial dysfunction. In summary, chronic daily sildenafil or tadalafil treatment could exert prolonged beneficial effects on endothelial dysfunction in patients with T2D (Desouza

et al., 2002) and such a benefit of chronic tadalafil dosing may last for more than 2 weeks after the discontinuation of treatment (Rosano et al., 2005).

3.2. Protection against ischemia-reperfusion injury in diabetic heart: Role of AMPK-SIRT1-PGC-1α cytoprotective signaling network

The diabetic myocardium is especially vulnerable to I/R injury (Van der Mieren et al., 2012; Miki et al., 2012) and is also known to be refractory to many cardioprotective modalities, such as ischemic preconditioning (Downey & Cohen, 2009) and ischemic postconditioning (Przyklenk et al., 2011; Zhu et al., 2012). Recent studies from our laboratory showed that chronic treatment with tadalafil significantly reduced infarct size in the T2D hearts (Koka et al., 2013; Varma et al., 2012).. A reduction in fasting glucose and triglyceride levels was also observed, whereas body weight was not altered by tadalafil treatment.

NO production is known to activate SIRT1, which is a histone deacetylase that regulates PGC-1α - a key regulator of mitochondrial biogenesis and co-activator of transcription factors impacting energy homeostasis. Treatment with tadalafil enhanced plasma NOx levels, myocardial SIRT1, PGC-1α expression and phosphorylation of Akt and AMPK in the diabetic hearts. Interestingly, these signaling changes were associated with attenuated mitochondrial dysfunction in T2D hearts as indicated by improved mitochondrial glutamate state 3 respiration rates and reduced ROS production from complex I (Koka et al., 2014) ultimately leading to the protection in diabetic heart (Figure 1).

3.3. PDE5 inhibition promotes antioxidant-like effects of in diabetic heart

Under T2D conditions, the myocardium is exposed to intense oxidative stress, which eventually leads to cardiac tissue injury and dysfunction. Chronic treatment with tadalafil caused significant suppression in ROS production, cardiac NADPH oxidase activity, lipid peroxidation, and oxidized glutathione (Koka et al., 2013). Moreover, tadalafil treatment attenuated myocardial expression of pRac1 and gp91phox, the subunits of NADPH oxidase enzyme. Furthermore, proteomic analysis revealed that glutathione S-transferase (GST) kappa 1 (GSTK1), a putative mitochondrial antioxidant enzyme - was significantly depressed in the T2D hearts (Koka et al., 2012). Interestingly, tadalafil treatment completely preserved GSTK1 in tadalafil-treated db/db mice, which was associated with improved cardiac GSH/GSSG ratio (Koka et al., 2012), indicating attenuated oxidative stress within the diabetic heart. These findings are compelling, because both the structure and the sub-cellular localization of GSTK1 (in mitochondria and peroxisomes) make this enzyme distinct from other cytosolic GSTKs due to its potential involvement in energy and lipid metabolism, two functions related to mitochondria and peroxisomes. Importantly, GSTK1 expression level is negatively correlated with obesity in mice as well as the human adipose tissues and therefore could be the determining factor in these metabolic disorders (Morel & Aninat, 2011). The role of GSTK1 in attenuating I/R injury in general and its potential contribution in PDE5 inhibitor-induced cardioprotection in T2D mice needs to be further evaluated.

3.4. Anti-inflammatory effects of PDE5 inhibitor in T2D hearts

Hyperglycemia in diabetic patients is associated with greater systemic inflammation (Aronson et al., 2004;Varo et al., 2003). This is because hyperglycemia increases binding of inflammatory cells to the endothelium (Morigi et al., 1998) and also enhances inflammatory cytokine production in monocytes. Diabetes is associated with greater inflammation and higher glucose levels in patients with acute coronary syndrome. Moreover, patients with both hyperglycemia and inflammation had worse outcomes (Ray et al., 2007). Interestingly, treatment of T2D db/db mice with tadalafil for 28 days showed significant reduction of TNF-α and IL-1β (Varma et al., 2012). At the same time, the anti-inflammatory cytokine IL-10 was significantly higher in the tadalafil-treated db/db mice compared to control. Taken together, these preclinical studies along with recent human data showing improvement in diabetic cardiomyopathy following chronic treatment with sildenafil (Giannetta et al., 2012) underscore the possibility that PDE5 inhibitors can be developed as possible therapeutic modality for diabetes-related cardiac and inflammatory complications.

4. PDE5 Inhibitors in Cancer

4.1. PDE5 expression in cancer cells: Potential anticancer effects of PDE5 inhibitors

Elevated PDE5 expression has been reported in multiple human carcinomas including colon adenocarcinoma, bladder squamous carcinoma, metastatic breast, prostate, pancreatic, and lung cancers as compared to adjacent normal tissues (Epstein & Hachisu, 1984; Joe et al., 2003; Lim et al., 2003; Piazza et al., 2001; Porst et al., 2001; Singer et al., 1976; Whitehead et al., 2003; Kumazoe et al., 2013). PDE5 has also been detected in many carcinoma cells, including colonic adenocarcinoma (SW480, HCT116, HT29, T84), breast cancer (HTB-26, MCF-7), lung cancer, bladder cancer and prostate cancer (LNCAP, PC-3), and leukemia (Thompson et al., 2000; Whitehead et al., 2003; Zhu et al., 2005). Since cGMP reduces cell growth and induces apoptosis, PDE5 enzyme has been suggested to be involved in tumor progression because its elevation occurs with increasing tumor grade and stage (Karami-Tehrani et al., 2012). Therefore, PDE5 selective inhibitors could be potent anticancer drugs with a novel mechanism of action (Figure 2). Accordingly, sildenafil and vardenafil induced caspase dependent apoptosis and antiproliferation in B-cell chronic lymphatic leukemia (Sarfati et al., 2003; Zhu et al., 2005). Another PDE5 inhibitor, exisulind (sulindac sulfone) and its higher affinity analogues also induced apoptosis and inhibited cell proliferation in colon tumor cells lines by activating the cGMP/PKG pathway and increasing phosphorylation of β-catenine (Lim et al., 2003; Liu et al., 2002). The transcriptional suppression of β-catenine inhibits oncogenic Wnt/β-catenine T-cell factor transcriptional activity, leading to down-regulation of cyclin D1 and survivin (Li et al., 2013). PDE5 knockdown by siRNA as well as tadalafil and sildenafil also inhibited the growth of colon tumor cells expressing high levels of PDE5 as compared with colonocytes (Li et al., 2013). Moreover, PDE5 inhibitors selectively induced apoptosis in breast tumor cells by attenuating Wnt/β-catenin mediated transcription in breast tumor cells with minimal effects on normal mammary epithelial cells (Tinsley et al., 2009; Tinsley et al., 2011).

4.2. Role in the treatment of radical prostatectomy-induced erectile dysfunction

All forms of prostate cancer therapy cause significant risk of erectile dysfunction (ED) due to trauma sustained by the cavernosal nerves (Rambhatla et al., 2008). PDE5 inhibitors significantly improved erectile function in men after radical prostatectomy (Mydlo et al., 2005; Ohebshalom et al., 2005; Schiff et al., 2006; Teloken et al., 2007). The prolonged and continuous administration of PDE5 inhibitors prevented fibrosis and loss of smooth muscle thereby reducing corporal veno-occlusive dysfunction (CVOD) following bilateral cavernosal nerve resection (Ferrini et al., 2006) (Kovanecz et al., 2008). Treatment with exisulind significantly suppressed the increase in PSA (prostate specific antigen) in all patients with prostate cancer following radical prostatectomy, compared with placebo (Goluboff et al., 2001). Additionally, early use of PDE5 inhibitor after prostate brachytherapy maintained erectile function at both 6 and 12 months (Pahlajani et al., 2010).

4.3. Enhancing the efficacy of chemotherapy with PDE5 inhibitors

PDE5 inhibitors also enhance the chemotherapeutic efficacy of anticancer drugs in prostate and other cancers. Sulindac sulfide and exisulind inhibited growth and induced apoptosis in both the androgen-sensitive (LNCaP) and androgen-insensitive (PC-3) human prostate cancer cell lines (Lim et al., 1999; Lim et al., 2003). Exisulind also suppressed the growth of human prostate cancer cells in an athymic nude mouse xenograft model (Goluboff et al., 1999). At a low dose, combination of colecoxib, a cyclooxygenase-2 (COX-2) inhibitor, with exisulind prevented prostate carcinogenesis, enhanced apoptosis (Narayanan et al., 2007), and exerted anti-inflammatory effects through reduced levels of COX-2, prostaglandin E2 and TNF-α. Therefore, a combination of potential agents at low doses is considered to be very efficacious in achieving excellent anticancer effects while minimizing overall toxicity compared with the use of individual agents at higher dose levels.

A recent study suggested that sildenafil interacted greater than additive fashion with a clinically relevant non-steroidal anti-inflammatory drug, celecoxib (Celebrex®, COX-2 inhibitor) to kill multiple tumor cell types including human glioma cells as well as their associated activated microglia (Booth et al., 2014c). The drug combination increased the levels of autophagy by inactivating mTOR and inducing endoplasmic reticulum (ER) stress responses in these cells. Sildenafil and celecoxib treatment also inhibited the growth of mammary tumors in vivo which was enhanced by the multiple sclerosis drug FTY720 (Fingolimod, Gilenya) that is known to suppress sphingosine-1-phosphate (S1P) signaling through S1P production and increasing the ceramide levels (Booth et al., 2014c). Sildenafil and tadalafil were also shown to interact with non-coxib celecoxib derivative OSU-03012 (lacking COX2-inhibitory activity) in killing of glioblastoma multiforme (GBM) cells by recruiting death receptor signaling (Booth et al., 2014b).

The combination of vardenafil with DOX in rats bearing brain tumors improved survival and reduced tumor size (Black et al., 2008). Oral administration of vardenafil or sildenafil increased the rate of transport of compounds across the blood-tumor barrier and improved the efficacy of DOX in brain tumors. The selective increase in tumor capillary permeability was mediated by an increase in tumor cGMP levels and increased vesicular transport and

was mediated by calcium-dependent potassium (K_{Ca}) channels, the putative effectors in cGMP signaling.

In prostate cancer cells, co-treatment with sildenafil potentiated the antitumor efficacy of DOX, while simultaneously reducing the risk of cardiomyopathy (Das et al., 2010). Proliferation of the prostate cancer cell lines, PC-3 and DU145, was reduced in a dose-dependent manner with DOX treatment. Sildenafil and DOX treatment enhanced expression of the pro-apoptotic proteins Bad and Bax while suppressing the expression of the anti-apoptotic proteins, Bcl-2 and Bcl-xL. Furthermore, combination treatment resulted in dephosphorylation of Bad, which may enhance Bad heterodimerization with Bcl-xL thereby promoting DOX-induced apoptosis. The ectopic overexpression of Bcl-xL in DU145 cells attenuated the synergistic effect of sildenafil and DOX on cell killing. Caspase-3 and -9 activities were also increased following sildenafil and DOX co-treatment while overexpression of dominant negative procaspase-9 in DU145 cells blocked the enhanced cell killing effect. Sildenafil also enhanced DOX-induced cancer cell killing through enhancing ROS generation. In contrast, sildenafil attenuated DOX-induced ROS generation in normal prostate cells preventing the increase in cell death. Treatment with sildenafil and DOX in mice bearing prostate tumor xenografts resulted in significant inhibition of tumor growth (Das et al., 2010). The reduced tumor size was associated with amplified apoptotic cell death and increased expression of activated caspase-3. The anti-tumor effect of sildenafil and DOX did not translate into increased cardiotoxicity; however, as this same combination ameliorated DOX-induced cardiac dysfunction. Another PDE5 inhibitor, Zaprinast, was also reported to reduce hypoxia-associated acquisition of resistance to DOX in prostate cancer cells and inhibited tumor growth in a xenograft model (Hamilton et al., 2013). The potential beneficial role of PDE5 inhibition in enhancing drug sensitivity was suggested to be due to activation of NO signaling in hypoxic cell populations.

Sildenafil also enhanced DOX-induced killing of ovarian cancer and sarcoma cells (Das et al., 2010). Interestingly, sulindac, selectively enhanced killing of cancer cells exposed to oxidizing agents via production of ROS (Resnick et al., 2009). On the other hand, low levels of sulindac induced a delayed preconditioning (cardioprotective) response against I/R injury in the heart through up-regulation of putative effectors of cardioprotection including iNOS and HSP27 (Moench et al., 2009).

PDE5 inhibitors enhance bladder and pancreatic cancer cell killing by interacting, in an on-target fashion, with DOX, mitomycin C, and Gemzar through increased death receptor signaling mediated by caspase 8, as well as increased autophagy mediated by receptor interacting protein 1 (RIP-1) pathways downstream of death receptors in bladder cancer (T24) cells (Booth et al., 2014a). The endogenous caspase 8 inhibitor, cFLIP-s, or the mitochondrial protective protein BCL-xL abolished the drug interaction as well as suppressed sildenafil-enhanced cell killing and chemotherapeutic toxicity. PDE5 inhibitors enhanced and prolonged the induction of DNA damage as judged by Comet assays along with histone H2AX and checkpoint kinase-2 (CHK2) phosphorylation. Sildenafil was also found to interact with multiple standard of care chemotherapeutic agents (vincristine, etoposide, and cisplatin) in an additive fashion to kill medulloblastoma cells by induction of DNA damage in a NO synthase-dependent pathway (Roberts et al., 2014). These results

suggested that sildenafil enhances chemotherapeutic efficacy through both death receptor and mitochondrial signaling as part of the combinatorial killing process.

4.4. Impact of PDE5 inhibitors in attenuation of multi-drug resistance

One of the major causes of chemotherapy failure in cancer treatment is multidrug resistance (MDR) as a result of overexpression of the ATP-binding cassette (ABC) transporters, such as P-glycoprotein (ABCB1/P-gp/MDR1), multidrug-resistance proteins (ABCCs/MRPs) and breast cancer resistant protein (ABCG2/BCRP). ABCB1 is the most well studied and important mediator of MDR (Ambudkar et al., 2003). It is responsible for resistance to a variety of drugs, including anthracyclines (Szakacs et al., 2006). These transporters use the energy of ATP hydrolysis to actively pump their substrate, in this case the chemotherapeutic agent, from cancer cells, thereby reducing drug accumulation (Dean et al., 2001;Gillet et al., 2007;O'Connor, 2007). Therefore, inhibiting these transporters would restore the sensitivity of drug-resistant cancer cells to chemotherapy leading to better efficacy in the treatment of cancer patients. Unfortunately, most of the transport inhibitors have not translated in the clinic due to unfavorable side effects, toxic pharmacokinetic interactions or simply because the magnitude of improvement over conventional chemotherapeutic agents is either non-significant or inconclusive (Szakacs et al., 2006). Interestingly, some PDE5 inhibitors have recently been found to inhibit the function of one or more ABC transporters. In one study, vardenafil was found to significantly reverse MDR in ABCB1-overexpressing cancer cells, and its efficacy was greater than that of tadalafil (Ding et al., 2011). Sildenafil also inhibited cell surface ABC transporters ABCB1 and ABCG2-mediated drug efflux, resulting in an increase in the intracellular concentrations of anticancer drugs and drug sensitivity (Shi et al., 2011). Thus sildenafil has the potential to improve the chemotherapeutic outcome of cancer patients by enhancing distribution and accumulation of chemotherapeutic drugs leading to better efficacy.

5. Concluding Comments and Future Perspective

Since our first report showing efficacy of sildenafil in reducing myocardial infarct size following ischemia/reperfusion injury (Ockaili et al., 2002), there has been tremendous growth of preclinical and clinical studies on the use of PDE5 inhibitors for cardiovascular disease and cancer. As reviewed above, preclinical studies with PDE5 inhibitors have shown promising results in these areas. Clinically, Revatio (sildenafil) and Adcirca (tadalafil) are being used for treatment of pulmonary arterial hypertension to improve exercise ability and slow down worsening changes in the physical condition of patients. Many clinical trials with PDE5 inhibitors have been completed or still ongoing which focus on the potential cardiovascular benefits (Clinical Trials.gov). For example, the clinical trial on sildenafil by Guazzi and colleagues (Guazzi et al., 2011) for possible treatment of diastolic heart failure demonstrated promising results In this trial, 45 patients with stable systolic heart failure were treated with sildenafil (50 mg three times daily) for 1 year showed improvements in functional capacity and clinical status. Another clinical investigation in 45 patients showed improvement in dysfunctional LV contraction in patients with non-ischemic, non-failing diabetic cardiomyopathy following chronic treatment sildenafil (Giannetta et al., 2012). This study showed that such an anti-remodeling mechanism was independent of vascular,

endothelial, or metabolic factor. In contrast, a multicenter, double-blind, randomized RELAX trial of 216 stable outpatients with heart failure and preserved ejection fraction did not show any significant improvement in exercise capacity or clinical status (Redfield et al., 2013). In addition, left ventricular diastolic function and pulmonary artery systolic pressure remained unaffected. In this trial, the patients were treated with sildenafil (20 mg three times daily for 3 months, followed by 60 mg three times daily for another 3 months). More recently, sildenafil (40 mg three times per day) was tested in a prospective, randomized, double-blind, placebo-controlled study on 70 patients with diastolic dysfunction after acute MI (Andersen et al., 2013). Again, sildenafil treatment group did not show any significant improvements in the predetermined primary end points (LV filling pressure at rest or during exercise). However, the meta-analysis of randomized, placebo-controlled trials suggested that PDE5 inhibitors had anti-remodeling properties and improved cardiac inotropism which was independent of afterload changes with a good safety profile (Giannetta et al., 2014). Based on this analysis and reproducibility of the results, the authors of this study recommended that PDE5 inhibitors could be reasonably offered to men with cardiac hypertrophy and early stage heart failure. Preclinical studies also suggest that sildenafil and other PDE5 inhibitors may enhance the sensitivity of certain types of cancer to standard chemotherapeutic drugs, including doxorubicin. However, a recent prospective cohort study revealed that sildenafil use may be associated with an increased risk of developing melanoma in men, although this study was not sufficiently powered to alter clinical recommendations (Li et al., 2014). Future ongoing or planned clinical investigations are expected to reveal whether PDE5 inhibitors have any influence in improving the efficacy of existing cancer therapies.

In summary, despite mixed results of clinical trials with PDE5 inhibitors in cardiac and cancer studies, there is continuing strong interest in exploring their new clinical uses. This is in part due to the overall safety record of PDE5 inhibitors and their clinical use for erectile dysfunction as well as pulmonary hypertension. We believe that future trials, which take into consideration the correct patient population based on their clinical indications need to be carefully planned to fully realize the benefits of PDE5 inhibitors for cardiovascular disease and cancer.

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Abbreviations

A1AR	adenosine A1 receptor
ABC transporters	ATP-binding cassette
ABCB1/P-gp/MDR1	P-glycoprotein
ABCG2/BCRP	breast cancer resistant protein

Ang-1	angiopoietin 1
ASCs	Adipose stem cells
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma extra-large
b-FGF	Basic fibroblast growth factor
calcineurin-NFAT	Calcineurin activates nuclear factor of activated T cell
cAMP	cyclic adenosine monophosphate
cFLIP-s	Fas-Associated Death Domain like interleukin-1 beta-converting enzyme (FLICE) inhibitory protein(c-FLIP) short form
cGMP	cyclic guanosine monophosphate
CHF	congestive heart failure
CHK2	checkpoint kinase-2
CO	carbon monoxide
COX-2	cyclooxygenase-2
CSE	cystathionine-γ-lyase
CVOD	corporal veno-occlusive dysfunction
DBMD	Duchenne/Becker muscular dystrophy
DMD	Duchenne muscular dystrophy
DOX	doxorubicin
ED	erectile dysfunction
eNOS	endothelial nitric oxide synthases
ER	Endoplasmic reticulum
ERK	extracellular signal-regulated kinases
FMD	flow-mediated dilatation
GAF	cGMP-binding PDEs, Anabaena Adenylyl cyclase, and Escherichia coli FhlA
GBM	glioblastoma multiforme
GSH/GSSG	ratio of reduced (GSH) and oxidized (GSSG) form of glutathione
GSK-3β	glycogen synthase kinase-3β
GSTK1	glutathione S-transferase (GST) kappa 1
H2S	hydrogen sulfide
HbA1c	glycated hemoglobin
HO-1	heme oxygenase

HSP27	heat shock protein 27
iNOS	inducible nitric oxide synthases
I/R	ischemia/reperfusion
IGF	Insulin-like growth factor
IL-1β	interleukin-1 beta
LV	Left ventricular
MDR	multidrug resistance
MI	myocardial Infarction
Δψm	mitochondrial membrane potential
mitoK$_{ATP}$	mitochondrial K_{ATP}
mitoKCa	mitochondrial Ca^{2+}-activated K
MnSOD	manganese superoxide dismutase
mTOR	mammalian target of rapamycin
NADPH	nicotinamide adenine dinucleotide phosphate-oxidase
NO	nitric oxide
PAG	dl-propargylglycine
PAH	pulmonary arterial hypertension
PDE5	phosphodiesterase 5
PGC-1α	peroxisome proliferator-activated receptor gamma coactivator 1-alpha
PKG	protein kinase G
PSA	prostate specific antigen
RIP-1	receptor interacting protein 1
ROS	reactive oxygen species
S1P	sphingosine-1-phosphate
sGC	soluble guanylate cyclase
siRNA	small interfering RNA
SIRT1	sirtuin 1
T2D	type 2 diabetes
TNF-α	tumor necrosis factor alpha
VEGF	vascular endothelial growth factor

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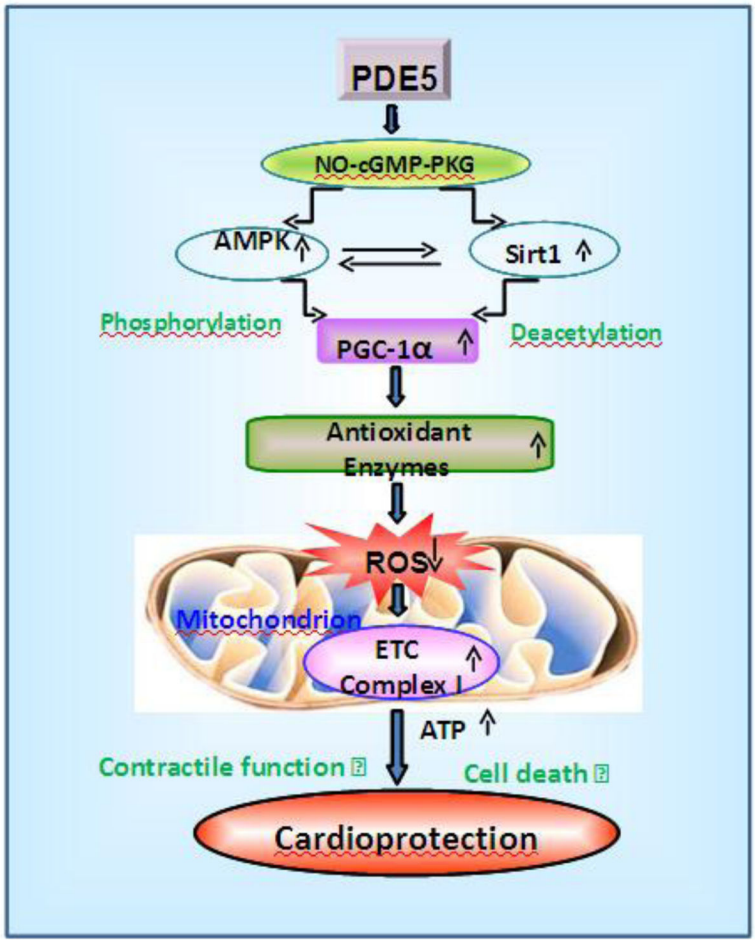

Figure 1.

Proposed mechanism of protection in diabetic heart by PDE5 inhibitors. PDE5 inhibitors activate PKG by prevention of cGMP breakdown as well as generation of NO-driven cGMP. PKG activates AMPK and SIRT1, and subsequently induces PGC-1α. PGC-1α induces antioxidant enzymes leading to reduction of ROS production as well as restoration of mitochondrial ETC complex I activity in diabetic heart. Restoration of mitochondrial function in diabetic heart improves cardiac function and subsequently leads to cardioprotection. PDE5: phosphodiesterase type 5, PDE5i: PDE5 inhibitor, NO: nitric oxide, cGMP: cyclic guanosine monophosphate, PKG: cGMP-dependent protein kinase, AMPK: AMP-activated protein kinase, SIRT1: sirtuin 1, PGC-1α: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha, ROS: reactive oxygen species, ETC; electron transport chain.

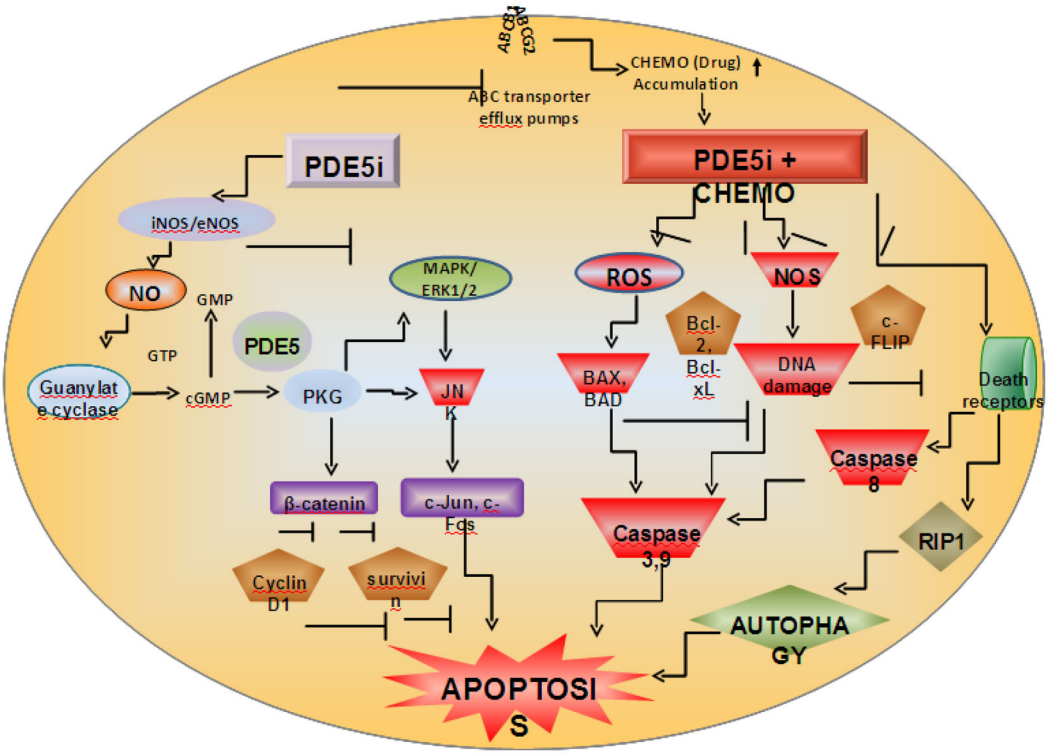

Figure 2.

Anti-tumor signaling pathways triggered by PDE5 inhibitors. PDE5 inhibitors induce NO signaling with enhanced formation of cGMP and resulting in PKG activation. PKG induces β-catenin, which attenuates multiple downstream survival proteins and also activates pro-apoptotic proteins leading to apoptosis. PDE5 inhibitors also enhance the effectiveness of multiple chemotherapeutics by increasing the intracellular accumulation of drugs through inhibition of ABC-transporter mediated efflux. In addition, chemosensitization with PDE5 inhibitors appears to be mediated by increased ROS generation, activation of pro-apoptotic (Bax, BAD) and autophagy related proteins (RIP1), inhibition of anti-apoptotic proteins (Bcl-2, Bcl-xL) and activation of Caspases-3, 9 and 8 ultimately driving the cells towards apoptosis. PDE5: phosphodiesterase type 5, PDE5i: PDE5 inhibitor, NO: nitric oxide, cGMP: cyclic guanosine monophosphate, PKG: cGMP-dependent protein kinase, ABC-transporter: ATP-binding cassette transporter, ROS: reactive oxygen species, Bax: Bcl-2-associated X protein, BAD: Bcl-2-associated death promoter protein, cFLIP: Fas-Associated Death Domain like interleukin-1 beta-converting enzyme (FLICE) inhibitory protein, RIP1: receptor-interacting protein 1, Bcl-2: B-cell lymphoma 2, Bcl-xL: B-cell lymphoma extra-large.