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Beyond Erectile Dysfunction: cGMP-Specific Phosphodiesterase 5 Inhibitors for Other Clinical Disorders

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Abstract

Cyclic guanosine monophosphate (cGMP), an important intracellular second messenger, mediates cellular functional responses in all vital organs. Phosphodiesterase 5 (PDE5) is one of the 11 members of the cyclic nucleotide phosphodiesterase (PDE) family that specifically targets cGMP generated by nitric oxide–driven activation of the soluble guanylyl cyclase. PDE5 inhibitors, including sildenafil and tadalafil, are widely used for the treatment of erectile dysfunction, pulmonary arterial hypertension, and certain urological disorders. Preclinical studies have shown promising effects of PDE5 inhibitors in the treatment of myocardial infarction, cardiac hypertrophy, heart failure, cancer and anticancer-drug-associated cardiotoxicity, diabetes, Duchenne muscular dystrophy, Alzheimer's disease, and other aging-related conditions. Many clinical trials with PDE5 inhibitors have focused on the potential cardiovascular, anticancer, and neurological benefits. In this review, we provide an overview of the current state of knowledge on PDE5 inhibitors and their potential therapeutic indications for various clinical disorders beyond erectile dysfunction.

INTRODUCTION

Cellular levels of the second messengers, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP), are maintained by a family of enzymes named cyclic nucleotide phosphodiesterases (PDEs) (1–3). The PDEs degrade the phosphodiester bond of 3′-5′-cAMP and 3′-5′-cGMP and convert them to their inactive forms: 5′-AMP and 5′-GMP, respectively (4). The PDEs are broadly classified into 11 different families, PDE1–PDE11, largely on the basis of their structure, function, and substrate specificity. PDE4, PDE7, and PDE8 hydrolyze cAMP exclusively, whereas PDE5, PDE6, and PDE9 hydrolyze cGMP (2). PDE1, PDE2, PDE3, PDE10, and PDE11 can hydrolyze both cAMP and cGMP.

PDE5, the focus of this review, encompasses several key features of PDEs, including the conserved carboxy-terminal end and a variable regulatory amino-terminal domain, which are present in cGMP-specific PDEs. The regulatory region of PDE5 contains two GAF domains (GAF-A and GAF-B) that control the catalytic activity and dimerization of the protein (5, 6). GAF domains are named on the basis of certain proteins in which they are found: cGMP-specific PDEs, adenylyl cyclases, and FhlA. Binding of cGMP to the GAF-A nucleotide pocket allosterically modulates the catalytic activity (7), and the C-terminal GAF-B domain plays a role in the dimerization of the PDE5 enzyme (8).

Humans express three PDE5 isoforms: PDE5A1, PDE5A2, and PDE5A3 (**Figure 1*a***). The variants may allow for differential control of *PDE5A* gene expression in various cells. In humans, the *PDE5A* gene is located on chromosome 4q26, a region that reportedly codes for three isoforms: PDE5A1, PDE5A2, and PDE5A3 (9, 10). PDE5A1 and PDE5A2 are expressed in most tissue types, whereas PDE5A3 is confined to smooth muscle cells. All three isoforms vary in their amino acid composition at the N terminus. PDE5 is abundantly expressed in the smooth muscle cells of the corpus cavernosum and cardiovascular system (5, 6). PDE5 is also expressed in vascular and visceral smooth muscle, skeletal muscle, platelets, kidney, lung, spinal cord, cerebellum, pancreas, prostate, urethra, and bladder (11, 12). Although PDE5 is present in coronary vascular smooth muscle cells (13), healthy myocardium does not express high levels of the enzyme (14). However, upregulation of PDE5 has been detected in congestive heart failure (HF) and right ventricular (RV) hypertrophy (15, 16).

Because cGMP levels modulate vascular tone, it is an obvious target for therapeutic intervention in multiple diseases. Sildenafil citrate was the first PDE5 inhibitor approved for the treatment of erectile dysfunction (ED). As shown in **Figure 1*b***, in addition to sildenafil, three other drugs are approved by the US Food and Drug Administration (FDA) for ED: tadalafil, vardenafil, and avanafil. Clinically available but non-FDA-approved PDE5 inhibitors for ED include lodenafil, udenafil, and mirodenafil; these drugs are available in some countries. When a man is sexually stimulated, either physically or psychologically, nitric oxide (NO) is released from noncholinergic, nonadrenergic neurons in the penis and from endothelial cells (17). NO diffuses into cells and activates soluble guanylyl cyclase, which converts GTP to cGMP, thereby stimulating protein kinase G (PKG), which initiates a protein phosphorylation cascade. This cascade results in a decrease in intracellular levels of calcium ions, ultimately dilating the arteries that bring blood to the penis and compressing the spongy corpus cavernosum (**Figure 2**). PDE5 inhibitor blocks enzymatic hydrolysis of cGMP in the corpus cavernosum, resulting in a similar outcome. Currently, the clinically approved indications of PDE5 inhibitors also include lower urinary tract symptoms (LUTSs) and pulmonary arterial hypertension (PAH). In addition, many preclinical studies have shown promising effects of PDE5 inhibitors in the treatment of myocardial infarction, cardiac hypertrophy, HF, cancer and anticancer-drug-associated cardiotoxicity, diabetes, Duchenne muscular dystrophy (DMD), Alzheimer's disease (AD), and other aging-related conditions.

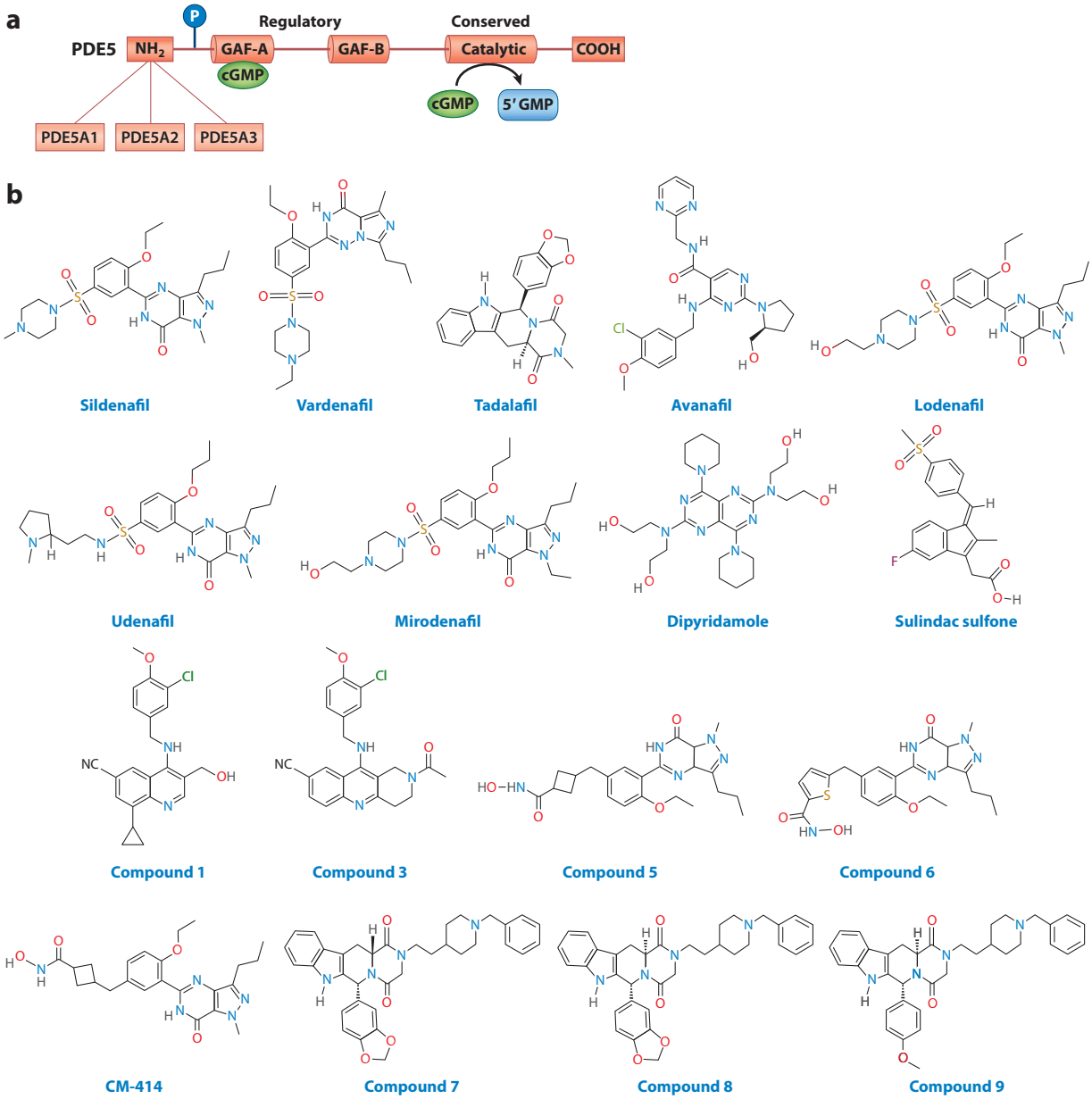

Figure 1

(*a*) Basic domain arrangement of phosphodiesterase 5 (PDE5) enzyme with three identified PDE5 isoforms (i.e., PDE5A1, PDE5A2, PDE5A3). Note that the key features of PDEs include the conserved carboxy-terminal end and a variable regulatory amino-terminal domain. The regulatory region of PDE5 contains two GAF domains (GAF-A and GAF-B) that control catalytic activity and dimerization of the protein. Binding of cyclic guanosine monophosphate (cGMP) to the GAF-A nucleotide pocket allosterically modulates the catalytic activity, while the C-terminal GAF-B domain plays a role in the dimerization of the PDE5 enzyme. (*b*) Chemical structures of the commonly used inhibitors of PDE5, which include those administered in humans as the US Food and Drug Administration–approved therapies for the management of erectile dysfunction, pulmonary arterial hypertension, and lower urinary tract symptoms. Compounds 1–9 are newly synthesized compounds for potential therapeutic use for neurodegenerative diseases. Compounds 1, 3, and 5–9 adapted with permission from Reference 182. CM-414 adapted with permission from Reference 199.

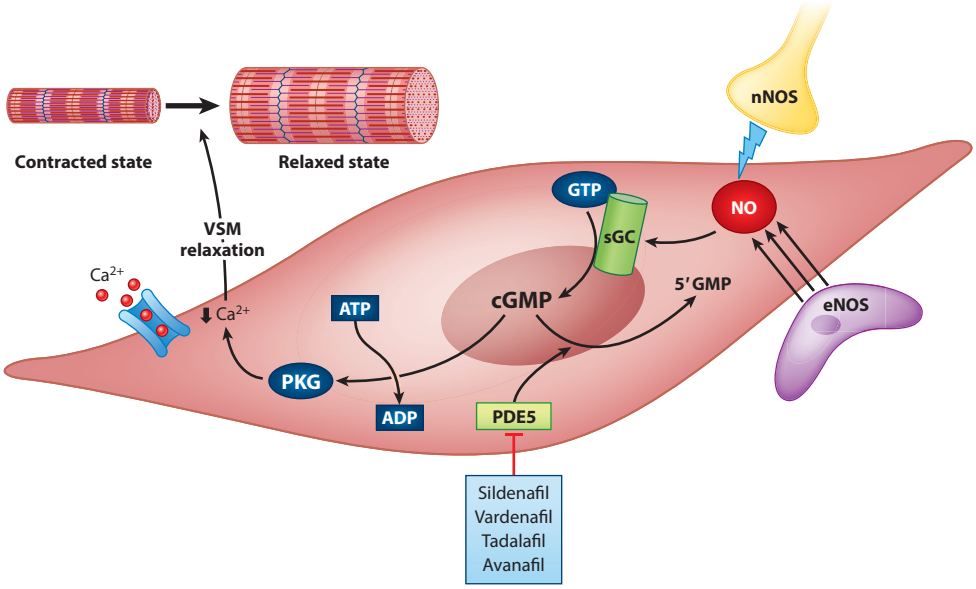

Figure 2

PDE5 as a therapeutic target for erectile dysfunction. Sexual stimulation releases NO from noncholinergic, nonadrenergic neurons in the penis and from endothelial cells. NO diffuses into cells and activates soluble GC, which converts GTP to cGMP. The cyclic nucleotide then stimulates PKG, which initiates a protein phosphorylation cascade, thereby decreasing intracellular levels of calcium ions, ultimately dilating the arteries that bring blood to the penis and compressing the spongy corpus cavernosum. PDE5 is the target for sildenafil and other PDE5 inhibitors for the treatment of erectile dysfunction. Abbreviations: cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate, eNOS, endothelial nitic oxide synthase; nNOS, neuronal nitric oxide synthase; NO, nitric oxide; PDE5, phosphodiesterase 5; PKG, protein kinase G; sGC, soluble guanylyl cyclase.

PDE5 IN PULMONARY ARTERIAL HYPERTENSION

PAH is a vascular disorder characterized by sustained elevation of mean pulmonary arterial pressure (mPAP) (≥25 mm Hg) in the presence of a pulmonary capillary wedge pressure (≤15 mm Hg) (18–20). Prolonged resistance in the lungs accompanied by enhanced vasoconstriction imposes burden on the right ventricle and triggers proliferation and narrowing of the pulmonary artery that carries blood to the lungs from the right ventricle. Constant elevation of mPAP in patients with PAH eventually leads to right heart failure and can result in death. Increased PDE5 expression is found in PAH patients (21) and sildenafil significantly improves pulmonary vasorelaxation (22–24). PDE5 is abundantly expressed in platelets and its inhibition mitigates platelet aggregation (25, 26). Insufficient NO-cGMP signal results in pulmonary thrombosis and platelet activation, which are common clinical manifestations of PAH (25, 26).

Sildenafil, tadalafil, and vardenafil increase pulmonary vasorelaxation in a dose- and time-dependent manner, with vardenafil having maximum effect at 40–45 min, sildenafil at 60 min, and tadalafil at 75–90 min (**https://www.clinicaltrials.gov/ct2/show/NCT00125918**). Results from the SUPER-1 (a double-blind study) and SUPER-2 (an open-label study) clinical trials involving PAH patients classified as WHO functional class II (who could perform ordinary activity with symptoms of dyspnea, fatigue, chest pain, or near syncope and comfortable at rest) or class III (marked limitation of activity and less than ordinary activity causing symptoms but

comfortable at rest) demonstrated beneficial effects of treatment with sildenafil. Hemodynamic parameters that include mPAP, pulmonary vascular resistance, and 6-minute walk distance were significantly improved. Moreover, 1-year survival was 96% in patients with idiopathic PAH, exceeding the anticipated survival of 71%. The REPLACE trial, aimed at replacement of riociguat, a soluble guanylate cyclase stimulant, with sildenafil, had a favorable outcome (**https://clinicaltrials.gov/ct2/show/NCT02891850**) and suggested PDE5 inhibition as an option for treating PAH patients with intermediate risk of 1-year mortality (27). Treatment with sildenafil enhanced the sensitivity of platelets to NO, which prevented platelet aggregation (28). Sildenafil infusion in utero improved PAH complications associated with impaired angiogenesis in persistent pulmonary hypertension of the newborn (29). Similarly, the administration of sildenafil in young adults born premature improved cardiac function, including RV flow measured by 4D flow magnetic resonance imaging (30). Sildenafil and tadalafil are FDA approved for the treatment of PAH and are used to reduce mortality either as monotherapy or in combination with prostacyclin analogs or endothelin-1 receptor blockers.

PDE5 IN ISCHEMIA/REPERFUSION INJURY

Ischemia is a condition in tissues and organs that is characterized by inadequate oxygen and nutrient supply following decreased blood flow. Paradoxically, the reperfusion of previously ischemic tissues and organs leads to additional tissue damage, a phenomenon called reperfusion injury. Myocardial reperfusion injury is also associated with percutaneous coronary interventions, stenting, coronary bypass surgery, and heart transplantation. In preclinical studies, rabbits treated with sildenafil before ischemia and reperfusion (I/R) had reduced myocardial infarct size, which was mediated by the opening of ATP-sensitive mitochondrial potassium (mito-K_{ATP}) channels (31). Vardenafil is 20-fold-more potent than sildenafil in inhibiting PDE5 (32). Therefore, a lower dose (i.e., 1/50-fold) of vardenafil reduced infarct size to the same degree as sildenafil does following I/R injury (33). Both drugs reduced infarct size when administered at reperfusion (34). Tadalafil, which has a longer half-life (35), also reduced infarct size and improved cardiac function following I/R in mice (36). Sildenafil had an antiarrhythmic effect when administered ≈20 h before myocardial ischemia in dogs. In these studies, the incidence of premature ventricular contractions, ventricular tachycardia, and ventricular fibrillation was reduced (37), which appeared to be an indirect effect of sildenafil because the serum concentrations of the drug may have fallen to low levels at the time of arrhythmia (38). Preconditioning of donor rats with vardenafil prior to explantation restored left ventricular (LV) function to the level without I/R injury after transplantation (39). Vardenafil also improved myocardial and endothelial function during cardiopulmonary bypass with hypothermic cardiac arrest (40). At the cellular level, sildenafil reduced necrosis and apoptosis in adult primary cardiomyocytes subjected to simulated ischemia/reoxygenation, suggesting its direct cytoprotective effect independent of the vascular/hypotensive effect (41).

Mechanistically, protection against I/R injury was mediated by NO signaling triggered through upregulation of inducible and endothelial nitric oxide synthase (iNOS and eNOS) (33, 41). The cardioprotective signaling with PDE5 inhibitors also involved the activation of adenosine A_1 receptor, protein kinase C, PKG, phosphorylation of extracellular signal–regulated kinase (ERK), and glycogen synthase kinase-3β in conjunction with an increase in Bcl-2, inhibition of apoptosis, inhibition of mitochondrial permeability transition pore, and upregulation of Sirt1 (42–47). PKG activation has also been linked to cardioprotection through the opening of mito-K_{ATP} channels (48, 49), which limits I/R injury through preservation of ATP and a decrease in Ca^{2+} influx into the mitochondria (**Figure 3**). It is proposed that PKG phosphorylates a target protein that shuttles the cardioprotective signal to protein kinase Cε, which resides in the intermembrane space of mitochondria (50). The basis for this model was that the combination of added PKG and cGMP

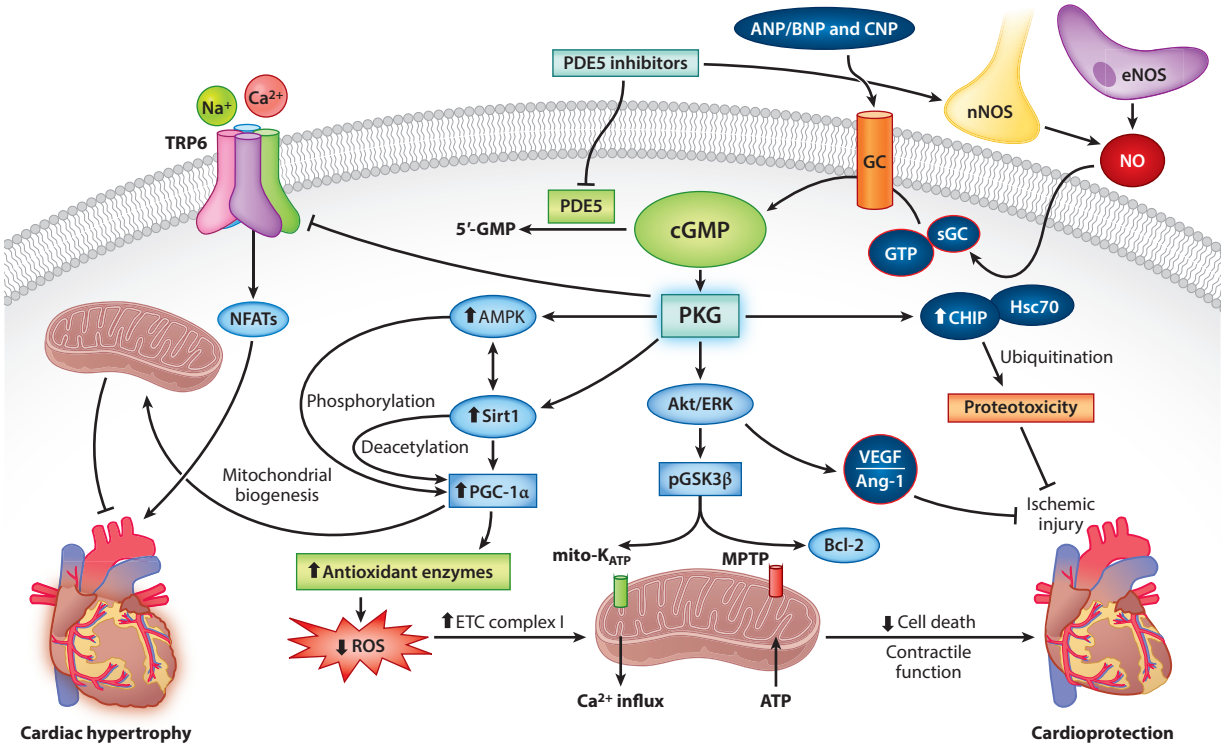

Figure 3

Cardioprotective pathways of NO-cGMP-PKG signaling in ischemic injury and cardiac hypertrophy. NO and cGMP generation by inhibition of PDE5 or activation of sGC triggers PKG signaling, which protects the heart by phosphorylating Akt, ERK1/2, and pGSK3β and inducing Bcl-2 as well as opening of mito-K_{ATP} channels. PKG activation also reduces ROS generation via AMPK–Sirt1–PGC-1α signaling, which protects the heart against cardiac hypertrophy and myocardial infarction. The antihypertrophic effect is also associated with activation of PKG, and its targets include regulator of G protein–coupled signaling-2 as well as calcineurin-NFAT and TRP6. PKG activation also provides posttranslational enhancement of protein quality control through facilitation of protein degradation via the proteasome and autophagy-lysosome-dependent pathways in ischemic heart. Abbreviations: AMPK, AMP-activated protein kinase; Ang-I, angiopoietin-1; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; CHIP, C-terminal Hsp70-interacting protein; CNP, C-type natriuretic peptide; ERK, extracellular signal-regulated kinase; GTP-guanosine triphosphate; HSc70, Heat shock cognate 71 kDa protein; mito-K_{ATP}, ATP-sensitive mitochondrial potassium channel; MPTP, mitochondrial permeability transition pore; NFAT, nuclear factor of activated T cells; NO, nitric oxide; PDE5, phosphodiesterase 5; pGSK3β, phospho-glycogen synthase kinase-3β; pGC, particulate guanylate cyclase; PGC-1α, peroxisome proliferator-activated receptor-gamma coactivator; pGSK3β, phosphorylated glycogen synthase kinase-3β; PKG, protein kinase G; ROS, reactive oxygen species; sGC, soluble guanylate cyclase; Sirt1, sirtuin 1; TRP6, transient receptor potential channel 6; VEGF, vascular endothelial growth factor.

activated mito-K_{ATP} in isolated mitochondria, whereas cGMP alone could not. In addition to NO, PKG-dependent generation of another gasotransmitter in cell signaling, hydrogen sulfide, also appeared to be involved in protection against I/R injury with the treatment of PDE5 inhibitor or by direct overexpression of PKG1α in the intact heart (36, 51).

Sildenafil also promoted angiogenesis following myocardial ischemia, as shown by increase in capillary and arteriolar density (52). The mechanisms involved phosphorylation of eNOS and Akt, activation of the VEGF–Ang-1 system (53), and mobilization of endothelial progenitor cells through a PKG-dependent HIF-1–VEGF pathway (54). Other studies have shown that PKG

activation provides posttranslational enhancement of protein quality control (**Figure 3**). The carboxyl terminus of Hsc70-interacting protein (CHIP) functions as an E3 ligase and cochaperone, which facilitates protein degradation via the proteasome and by autophagy-lysosome-dependent pathways (55, 56). CHIP is phosphorylated by PKG at a conserved Ser20 (S20 in mouse, S19 in human), which results in enhanced CHIP functionality by increasing its posttranslational half-life and protein interaction with Hsc70. Genetic loss of CHIP is associated with poorer cardiac responses to hemodynamic or ischemic stress (57, 58). Downregulation of PKG activity lowered CHIP-S20 phosphorylation and protein and exacerbated proteotoxicity and cardiac dysfunction following ischemia in mice (59). Conversely, CHIP-S20E knockin mice had improved clearance of ubiquitinated proteins and protection against ischemia. These studies suggested another mechanism by which PKG and possibly PDE5 inhibitors may have a role in attenuation of myocardial I/R injury.

PDE5 INHIBITORS IN HEART FAILURE

Myocardial infarction and other stimuli, including pressure and volume overload, trigger a complex process of myocardial derangements, including myocyte hypertrophy and loss, ventricular wall thinning and dilatation, and fibrosis (60, 61). Although these pathophysiological outcomes initially increase the capacity of the heart for compensation, they lead to maladaptive remodeling with a progressive impairment of contractile function and eventually HF. In HF with reduced ejection fraction (HFrEF), the LV systolic dysfunction leads to tissue hypoperfusion, which then causes oxidative stress and inflammation. HF with preserved ejection fraction (HFpEF) results from a complex interplay of risk factors, such as obesity, hypertension, cardiac aging, and loss of cardiovascular reserve, among others. A deficiency of cGMP has adverse effects on the heart, kidneys, and vessels, which may contribute to disease progression in HFrEF and HFpEF (62). In a mouse model of ischemic cardiomyopathy, treatment with sildenafil following myocardial infarction improved cardiac function and survival and decreased cell death in the myocardial infarction border zone (63, 64). Sildenafil also reversed transaortic constriction–induced hypertrophy and improved ejection fraction in HF (65), and attenuated LV remodeling and exercise intolerance following chronic mitral regurgitation (66). The antihypertrophic effect was associated with PKG activation; its targets included regulator of G protein–coupled signaling-2, calcineurin-NFAT, and transient receptor potential channel 6 (TRP6) (**Figure 3**). TRP6 is one of the nonselective and nonvoltage-gated ion channels that convey signaling information linked to a broad range of sensory inputs (67).

Activation of PKG by sildenafil also affects mitochondrial function during HF (68). Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is a transcriptional coactivator that is critical for mitochondrial biogenesis, ATP generation, ROS detoxification, and angiogenesis (69). PGC-1α is a target for several signaling pathways, including NO-cGMP-PKG (70, 71). Treatment with sildenafil maintained mitochondrial function by replenishing PGC-1α and inhibited adverse remodeling in later stages of HF through activation of PKG (68). Despite these promising results, other studies suggested that PKG activity does not modulate cardiac hypertrophy in the normal or failing heart (72, 73). Likewise, LV hypertrophy following transaortic constriction was not altered in the cardiac-specific PKG knockout mouse (72). Treatment with sildenafil also failed to decrease cardiac hypertrophy but decreased fibrosis following angiotensin II infusion, suggesting that PDE5 inhibition in cells other than cardiac myocytes and smooth muscle was responsible for the antifibrotic effect (73).

In clinical trials, treatment with sildenafil improved exercise capacity and ventricular function in HFrEF (74–76). However, mixed results were found in HFpEF, with the largest study failing to demonstrate clinical benefit (74, 77). A more recent single-center, randomized study of

50 patients demonstrated that 6-month therapy with sildenafil was associated with an increase in exercise capacity in patients with HFpEF (78). Patients treated with sildenafil also had a decrease in pulmonary vascular resistance and an improvement in RV systolic and diastolic function. These studies suggest that the role of sildenafil should be further examined in randomized trials in selected patients with HFpEF (78).

PDE5 IN DUCHENNE AND BECKER MUSCULAR DYSTROPHY

DMD is a progressive and fatal genetic disorder of muscle degeneration. Patients with DMD and Becker muscular dystrophy (BMD) carry mutations in the X-linked dystrophin gene (79). Dystrophin supports the plasma membrane of skeletal myofibers and cardiomyocytes. The loss of dystrophin leads to progressive muscle wasting, HF, and weakness, which are generally more severe in DMD than in BMD. Dystrophin-deficient mice (*mdx* mice) have cardiac dysfunction with a decrease in diastolic function followed by systolic dysfunction later in life. Some patients also suffer from cognitive impairment (80). Stimulation of cGMP synthesis by overexpression of cardiac-specific neuronal NOS (nNOS) reduced impulse-conduction defects in *mdx* mice (81, 82). Moreover, treatment with sildenafil or tadalafil in *mdx* mice reduced muscle damage (83), reversed cardiac dysfunction, and delayed the onset of cardiomyopathy (84, 85) with improved diaphragm contractility and reduced fibrosis (86). A clinical trial of sildenafil or tadalafil in 10 patients with DMD showed enhanced blood flow and improved exercise-induced functional sympatholysis in skeletal muscle. Tadalafil also alleviated muscle ischemia in eight of nine patients with BMD (87). However, in a Phase III trial, DMD treated with tadalafil failed to improve the 6-minute walk distance after 48 weeks of treatment (88). Further analysis in a subgroup of less disabled DMD boys revealed that the total and shoulder-level upper limb scores had lesser decline following treatment with tadalafil compared with placebo (88, 89). Thus, it appears that cGMP enhancement with PDE5 inhibitors was beneficial but still under the critical threshold of clinical benefit.

PDE5 IN ENDOTHELIAL DYSFUNCTION, METABOLISM, AND DIABETES

Metabolic syndrome comprises several risk factors, including abdominal obesity, dyslipidemia, hypertension, and insulin resistance (90). It is associated with increased risk of multiple chronic diseases, including cardiovascular disease, type 2 diabetes, arthritis, chronic kidney disease, cancer, and all-cause mortality (91–93). Impairment of NO bioavailability and the cGMP-PKG signaling cascade can lead to endothelial dysfunction (94) and decreased insulin utilization in high-fat diet–fed mice with insulin resistance (95). The browning of adipocytes in white fat depots of adipose tissue improves insulin sensitivity and metabolic syndrome (96–98). Treatment with sildenafil increased adipogenesis in the 3T3-L1 adipocyte cell line through the cGMP-PKG pathway (99) and induced browning of adipose tissue with increased expression of uncoupling protein 1 (UCP1) and PGC-1α (99) (**Figure 4**). The thermogenic capacity of brown adipose tissue was reduced in PKG knockout mice with decreased expression of UCP1 and mitochondrial content (100). PKG also controlled insulin signaling in brown adipose tissue by inhibiting RhoA activity and Rho-associated kinase, thereby removing its inhibitory effects on insulin receptor substrate-1 and activating the downstream PI3-kinase–Akt cascade (**Figure 4**). Conversely, mice overexpressing PKG were resistant to diet-induced obesity with increased insulin sensitivity, energy expenditure, browning of adipose tissue, mitochondrial biogenesis, and UCP1 expression (101). Likewise, treatment with tadalafil improved insulin sensitivity, lowered circulating lipids, increased expression of thermogenic markers, attenuated ROS generation, and promoted preadipocyte differentiation toward a metabolically healthy phenotype (102). Short-term treatment with the PDE5 inhibitor

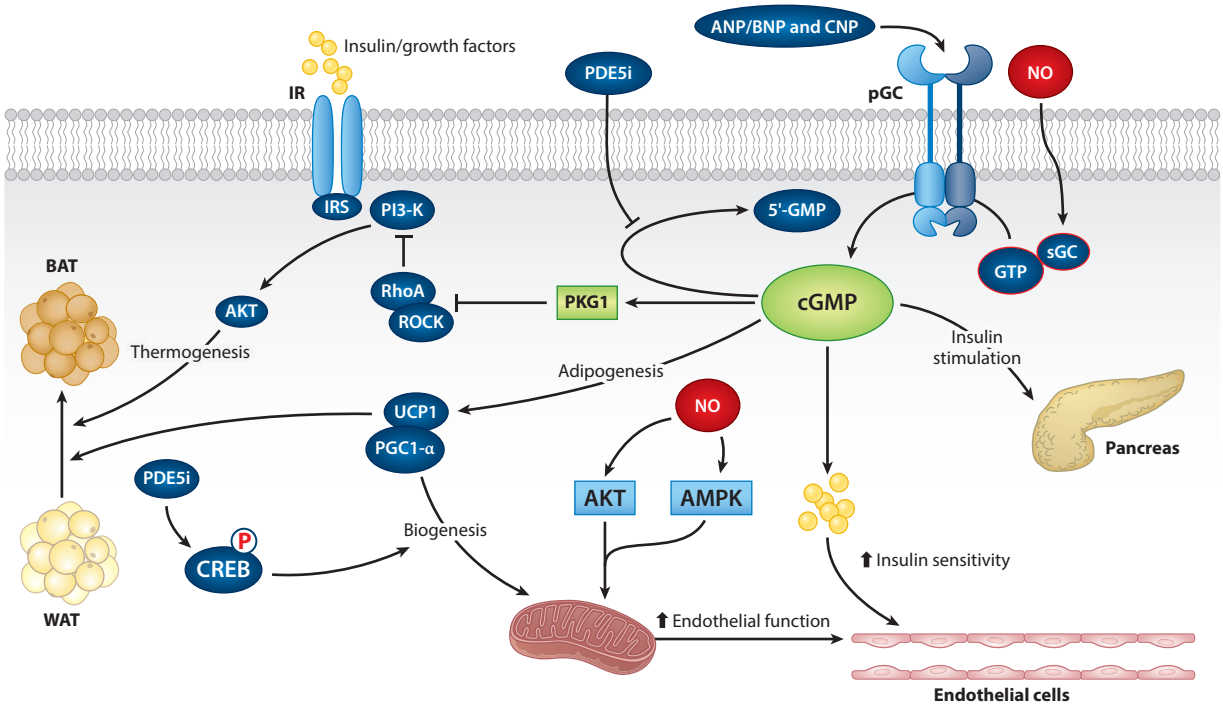

Figure 4

Role of PDE5 in regulation of metabolic diseases. Maintenance of cGMP-PKG pathway by PDE5 inhibition is critical for improving endothelial and metabolic function and browning of adipose tissue of WAT through increased expression of UCP1 and PGC-1α, leading to mitochondrial biogenesis. PKG controls insulin signaling in BAT by inhibiting RhoA activity and ROCK, thereby removing its inhibitory effects on IRS-1 and activating the downstream PI3-kinase–Akt cascade. Abbreviations: ANP, atrial natriuretic peptide; BAT, brown adipose tissue; BNP, brain natriuretic peptide; cGMP, cyclic guanosine monophosphate; CNP, C-type natriuretic peptide; CREB; cAMP-response element binding protein; IR, insulin receptor; IRS-1, insulin receptor substrate-1; PDE5, phosphodiesterase 5; PDE5i, PDE5 inhibitor; pGC, particulate guanylate cyclase; PGC-1α, peroxisome proliferator-activated receptor γ coactivator-1α; PI3K, phosphatidylinositol-3 kinase; PKG, protein kinase G; ROCK, Rho-associated kinase; UCP1, uncoupling protein 1; WAT, white adipose tissue.

udenafil reduced body weight, visceral fat mass, and appetite primarily through reduction of leptin plasma levels in mice fed a high-fat diet (103).

Another major issue is that the diabetic myocardium is vulnerable to I/R injury (104, 105) and refractory to many cardioprotective modalities, including pre- and postconditioning (106). The use of PDE5 inhibitors in patients with type 2 diabetes and cardiovascular risk factors was associated with reduced mortality (107). In addition, PDE5 inhibitors prevented post–myocardial infarction complications and future cardiovascular events in these patients. In a clinical trial of 59 men with diabetic cardiomyopathy, 3 months of treatment with sildenafil exerted significant anti-remodeling effect with improvement in circulatory biomarkers, including monocyte chemotactic protein-1 and transforming growth factor-β, compared with placebo (108). In male patients with type 2 diabetes, chronic treatment with vardenafil produced sustained improvements in endothelial parameters and restored testosterone levels in men with hypogonadism (109). In preclinical studies, treatment of *db/db* diabetic mice with tadalafil reduced myocardial infarct size and improved mitochondrial function and inflammation following I/R injury (110–112). Treatment with tadalafil in these mice also enhanced plasma NO levels, myocardial Sirt1, PGC-1α

expression, and phosphorylation of Akt and AMP-activated protein kinase (AMPK) (113) (**Figure 4**). In a model of streptozotocin or high-fat-diet-induced diabetes mellitus or the related cardiomyopathy, vardenafil improved cardiac function via activation of the NO-cGMP-PKG pathway (114, 115). Akt3, a serine/threonine kinase of the Akt family, is required for angiogenic responses, independent of Akt1 (116). Akt3 indirectly affects PGC-1α by controlling its nuclear retention via the regulation of CRM-1, the major nuclear export receptor, which increases nucleus-encoded mitochondrial gene expression (117). The perturbation of the Akt3–PGC-1α pathway or inhibition of electron transport by paraquat in endothelial cells produces mitochondrial dysfunction and decreased angiogenesis. Treatment with sildenafil rescued mitochondrial stress via direct binding of the transcription factor cAMP responsive element binding protein (CREB) to the PRC promoter, thereby increasing mitochondrial biogenesis and leading to increased angiogenesis (116). Thus, collectively, targeting the NO-cGMP-PKG pathway with PDE5 inhibitors may be a potential therapeutic strategy for the management of metabolic syndrome, diabetes, and associated cardiovascular disorders.

PDE5 IN CANCER

cGMP regulates cancer cell growth, adhesion, and the tumor microenvironment, such as blood flow, angiogenesis, inflammation, and immune response (118). Increased PDE5 expression has been associated with tumorigenesis in multiple cancer types (e.g., colon, bladder squamous, pancreatic, prostate, lung, or breast carcinomas) (119–126) and in many cancer cells, including colonic adenocarcinoma (SW480, HCT116, HT29, T84), breast cancer (HTB-26, MCF-7), lung cancer, bladder and prostate cancer (LNCAP, PC-3), and leukemia (126–128). PKG expression and cGMP levels are reduced in cancer cells and malignant tumors due to increased activation of PDE5, compared to its activity in normal or surrounding nonneoplastic tissues (120, 121). Thus, restoration of intracellular cGMP signaling with PDE5 inhibitors to inhibit proliferation, motility, and invasion of certain cancer cells is a rational approach for cancer therapy.

The anticancer effects of the PDE5 inhibitors on different types of cancers have been demonstrated (summarized in **Table 1**). The nonsteroidal anti-inflammatory drug exisulind (sulindac sulfone), which is also a PDE5 inhibitor (**Figure 1*b***), augments apoptosis and blocks tumor cell proliferation in urinary bladder tumor, metastatic breast cancer, non-small-cell lung cancer, and colon tumor cells lines through activation of the cGMP-PKG pathway and inhibition of the oncogenic activity of β-catenin (123, 126, 127, 129) (**Figure 5**). Sildenafil and vardenafil promote caspase-dependent apoptosis in B cell chronic lymphatic leukemia and human colon cancer cells by inhibiting the hydrolysis of cGMP (124, 128). Sildenafil inhibits colonic tumorigenesis by regulating the inflammatory microenvironment via inhibiting the infiltration of myeloid-derived suppressor cells (MDSCs) in colonic tissue (130, 131). Treatment with sildenafil neutralized the inflammatory tumor microenvironment by attenuating the immunosuppressive activities of MDSC, which is a therapeutic strategy for pancreatic ductal adenocarcinoma and melanoma (132, 133). Activation of cGMP-PKG signaling with tadalafil induced apoptosis and suppressed tumor growth in head and neck squamous cell carcinoma (HNSCC) xenografts in athymic mice (134). Randomized clinical trials in patients with HNSCC (**https://clinicaltrials.gov/ct2/show/NCT00843635**, **https://clinicaltrials.gov/ct2/show/NCT00894413**) demonstrated efficacy of tadalafil to augment tumor-specific immunity with suppression of circulating MDSC and regulatory T cell (Treg) populations (135, 136).

The prolonged use of sildenafil for ED has been associated with increased risk of developing melanoma and other skin cancers (137–142). Use of PDE5 inhibitors for ED following radical prostatectomy has been associated with worse outcome based on multivariate analysis and

Table 1 List of various types of cancers responsive to PDE5 inhibitors and their mechanism(s) of action as either single agent or adjuvant therapy with other cancer drugs

Cancer type(s)	Drug(s)	Mechanism(s) of action (reference)
Single agent		
Breast cancer	Sildenafil/Y-27632 Exisulind (sulindac)	Inactivated Rho GTPase signaling (119) Activated the cGMP-PKG pathway and induced apoptosis (125, 129)
B cell chronic lymphocytic leukemia	Sildenafil Vardenafil	Activated caspase-dependent apoptosis (124, 128)
Urinary bladder tumor Nonsmall cell lung cancer Colon tumor	Exisulind	Activated the cGMP-PKG pathway and apoptosis (123, 126) Inhibited the oncogenic activity of β-catenin (127)
Colon cancer Pancreatic ductal adenocarcinoma Melanoma	Sildenafil	Inhibited infiltration in MDSC (130, 131)
Head and neck squamous cell carcinoma	Tadalafil	Activated cGMP-PKG signaling (134) Augmented tumor-specific immunity (135) Suppressed circulating MDSC and Treg populations (136; **https://clinicaltrials.gov/ct2/show/NCT00843635**, **https://clinicaltrials.gov/ct2/show/NCT00894413**)
Melanoma and other skin cancers	Sildenafil	Targeted oncogenic BRAF (137) Acted through MEK and increased cGMP (138) Induced melanoma cell invasion (139, 142)
Adjuvant therapy		
Prostate cancer	Sildenafil	Upregulated the NO-cGMP-JNK pathway and enhanced cell killing and apoptosis (122) Increased doxorubicin-induced cytotoxicity and antitumor potency, and preserved cardiac function (151) Potentiated the antiproliferative activity of androgen deprivation therapy and stabilized androgen receptor (160)
Prostate cancer Bladder cancer Pancreatic cancer	Sildenafil	Enhanced cytotoxicity of mitomycin C, doxorubicin, cisplatin, and gemcitabine by inducing death receptor Fas (APO-1/CD95)-mediated apoptosis (152–154)
Medulloblastoma Breast cancer Hepatoma Colorectal cancer Lung cancer Glioblastoma cells	Sildenafil or tadalafil	Augmented cytotoxicity of other standard chemotherapy drugs (doxorubicin, cisplatin, oxaliplatin, vincristine, etoposide, and celecoxib) by enhancing drug uptake (152, 154–156)
Brain tumor	Sildenafil Vardenafil	Increased tumor capillary permeability (161) Reduced drug resistance inhibition of ABC transporters, ABCB1/ABCG2/ABCC5/ABCC10, and drug efflux (163–165) Increased intracellular concentrations of anticancer drugs (162)
Lung cancer Brain lymphoma	Vardenafil Tadalafil	Increased trastuzumab accumulation in tumors (156) Enhanced immunotherapeutic efficacy of rituximab by improving the microvascular permeability (166, 167)

Abbreviations: ABC, ATP-binding cassette; cGMP, cyclic guanosine monophosphate; MDSC, myeloid-derived suppressor cell; MEK, mitogen-activated protein kinase kinase; NO, nitric oxide; PDE5, phosphodiesterase 5; PKG, protein kinase G; Treg, regulatory T cell.

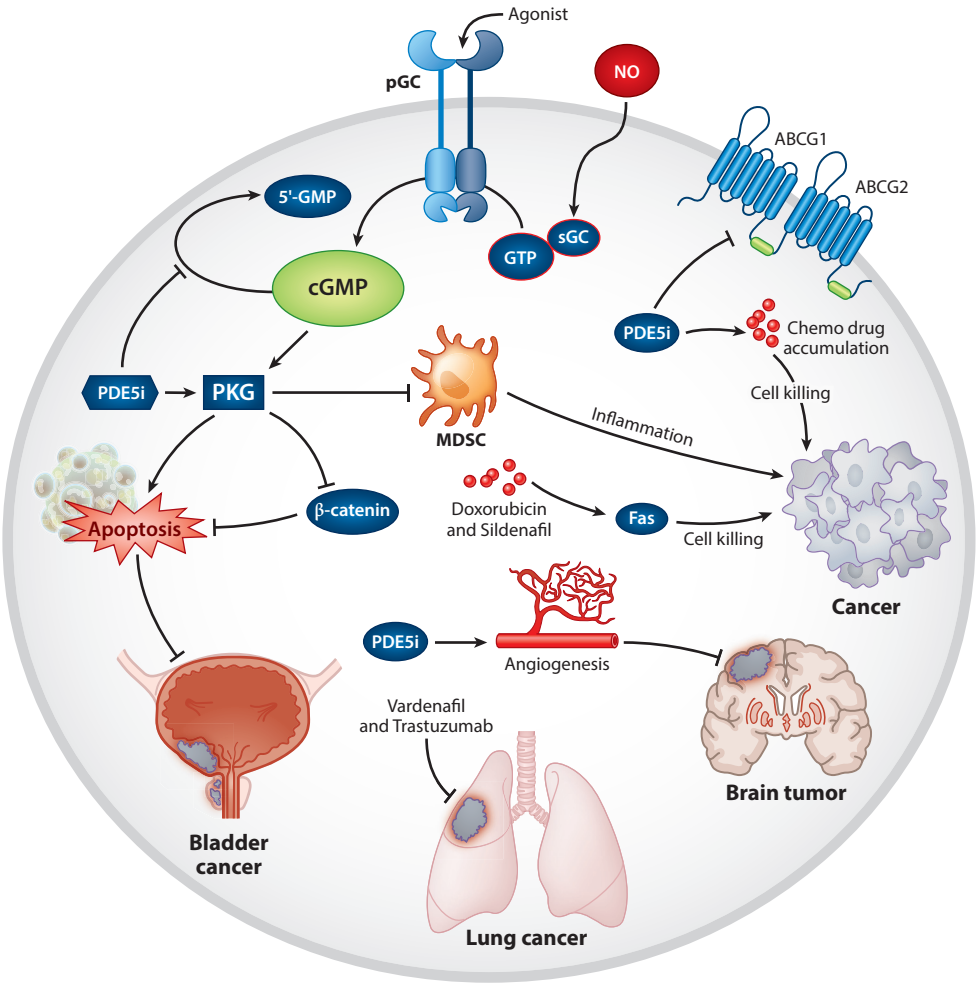

Figure 5

PDE5 inhibitors and anticancer signaling. The NO-cGMP signaling triggered by PDE5 inhibition inhibits β-catenin and increases killing of multiple types of cancers. PDE5 inhibitors also enhance the effectiveness of multiple chemotherapeutics, including doxorubicin, by increasing their intracellular accumulation through inhibition of ABC transporter–mediated efflux. Abbreviations: ABCG1/2, ATP-binding cassette subfamily G 1/2; cGMP, cyclic guanosine monophosphate; MDSC, myeloid-derived suppressor cell; NO, nitric oxide; PDE5, phosphodiesterase 5; PDEi, PDE inhibitor; PKG, protein kinase G; pGC, particulate guanylate cyclase; sGC, soluble guanylate cyclase.

propensity score matching (143). Other studies supported the association of PDE5 inhibitors with recurrence-free survival after radical prostatectomy (144, 145) and a protective effect against primary prostate cancer (146). A Swedish population-based cohort study found that PDE5 inhibition was associated with reduced risk of tumor progression/metastasis and mortality among patients with colorectal cancer, especially those receiving surgery (147).

PDE5 inhibitors can enhance the efficacy of chemotherapeutic drugs by targeting key pathways in a synergistic or additive manner (42, 148–150). Sildenafil increased doxorubicin-induced cytotoxicity in several cancer cell lines and antitumor potency in mice bearing prostate tumors

while ameliorating cardiac dysfunction (151). Sildenafil also enhanced the lethality of mitomycin C, doxorubicin, cisplatin, and gemcitabine via induction of Fas (APO-1/CD95)-mediated apoptosis in prostate, bladder, pancreatic, and other cell lines (152–154). Cotreatment with sildenafil or tadalafil augmented cytotoxicity of other standard chemotherapeutic drugs, including vincristine, cisplatin, etoposide, and celecoxib, in medulloblastoma, breast, hepatoma, colorectal cancer, and glioblastoma cells (152, 154, 155). In lung cancer cells, PDE5 inhibitors (dipyridamole, vardenafil, and/or sildenafil) increased the efficacy of doxorubicin, cisplatin, and oxaliplatin by enhancing endocytosis-mediated cellular drug uptake (156). A clinical trial (**https://clinicaltrials.gov/ct2/show/NCT01375699**) evaluated the cardioprotective effect of sildenafil after doxorubicin chemotherapy in patients with breast cancer (157). Although sildenafil was found to be safe, there was no evidence of significant improvement of cardiac function after treatment with doxorubicin. However, this trial was conducted in a limited number of patients (primarily women) and received less cumulative doxorubicin than doses associated with significant cardiac toxicity (i.e., 300 mg/m^2).

Docetaxel, a drug that is used to treat breast cancer, HNSCC, and stomach cancer, also has an important role in the management of advanced prostate cancer. However, more than half of patients do not respond to docetaxel, and good responders frequently experience significant cumulative toxicity, thereby limiting dose duration and amount (158, 159). Sildenafil synergistically enhanced docetaxel efficacy by affecting prostate cancer cell growth and inducing apoptosis in androgen receptor–positive human and mouse prostate cancer cell lines by triggering NO-cGMP-PKG-JNK signaling (122). In these studies, cotreatment with sildenafil augmented antitumor efficacy of docetaxel in vivo and reduced the growth of prostate-derived tumoroids from PTEN conditional knockout mice. Tadalafil also potentiated antiproliferative activity of androgen deprivation therapy in human prostate cancer cells by stabilizing the androgen receptor (160).

The blood-brain barrier significantly impedes delivery of therapeutic concentrations of chemotherapy to brain tumors. Sildenafil and vardenafil selectively increased tumor capillary permeability and efficacy of chemotherapy in rat models of brain tumor (161). ATP-binding cassette (ABC) protein transporters, including ABCB1, ABCC1, and ABCG2, mediate cellular efflux of chemotherapeutics and decrease intracellular concentration of drugs. Sildenafil and vardenafil reduced chemotherapeutic drug resistance by directly inhibiting ABCB1/ABCG2/ABCC5/ABCC10-mediated drug efflux, thereby increasing the intracellular concentrations of anticancer drugs and ensuing drug sensitivity (162–165).

Trastuzumab is a monoclonal antibody that is used to treat patients with breast, lung, or prostate cancer that overexpresses human epidermal growth factor receptor 2 (HER-2). Adjuvant treatment with vardenafil significantly increased accumulation of trastuzumab in tumors and enhanced its antitumor effect in a xenograft mouse model of lung cancer (156). Tadalafil also enhanced immunotherapeutic efficacy of rituximab (a chimeric anti-CD20 monoclonal antibody) by improving microvascular permeability in mouse brain lymphoma (166, 167). Combination therapy with anticancer drugs and PDE5 inhibitors also ameliorated drug resistance while reducing tumor growth and metastatic potential, arresting mitotically active cells, and inducing apoptosis. Thus, there are potential benefits of using combination therapy of PDE5 inhibitor and certain cancer drugs to improve outcomes in patients with cancer. Further investigations are needed to understand the molecular mechanisms of PDE5 inhibitors on specific types of cancers.

PDE5 IN AGING-RELATED DISEASES AND CONDITIONS

Testosterone controls the expression of PDE5. Accordingly, androgen supplementation improves therapeutic response to PDE5 inhibitors in hypogonadal subjects (168). The NO-cGMP

signaling pathway has been implicated in reducing testicular steroidogenesis during aging (169). Aging-associated low testosterone has been observed along with increased nitrite levels in the circulation, increased cGMP accumulation in testicular interstitial fluid, progressive atrophy of testicular seminiferous tubules, enlargement of interstitial area, and reduced steroidogenic capacity of Leydig cells (169). Long-term treatment with sildenafil in older rats normalized serum testosterone/nitrite levels and improved Leydig cell steroidogenic capacity. Mitochondrial dysfunction is responsible for decreased Leydig cell activity and lower testosterone production during aging (170). Aging-related accumulation of cGMP in Leydig cells was associated with mitochondrial dysfunction, reduced ATP and steroid production, lower O_2 consumption, increased mitochondrial abundance and mitochondrial DNA copy number, and decreased expression of mitochondrial biogenesis-regulating genes (i.e., *Ppargc1a/PGC-1α–Tfam–Nrf1/NRF1*). Acute in vivo PDE5 inhibition enhanced cGMP and stimulated testosterone production but reduced ATP production in Leydig cells from adult, middle-aged, and old rats. By contrast, long-term PDE5 inhibition decreased cGMP signaling but improved mitochondrial function/dynamics in Leydig cells from old rats (170).

Age-dependent effects on PDE5-cGMP signaling in cardiac muscle (171) and bones (172) were observed. Aging mice exhibit cardiac structural abnormality and contractile dysfunction, along with augmented oxidative and endoplasmic reticulum stress, which are ameliorated by exercise via activation of cGMP and suppression of PDE5 in the myocardium (171). PDE5 expression in mouse and human bones, as well as in sympathetic neurons (172), was identified. There appears to be a balance between peripheral and central actions of PDE5 inhibitors on bone formation and the antiresorptive and osteo-protective actions.

PDE5 is highly expressed in human hair follicles and dermal papilla cells; sildenafil enhanced their proliferation by upregulating VEGF and platelet-derived growth factor, leading to hair growth (173). Thus, PDE5 inhibitors may have a role in promoting hair growth and treating age-associated alopecia.

Endothelial dysfunction is often associated with vascular pathologies, such as atherosclerosis and diabetic vasculopathy. Reduced cGMP responsiveness and vasorelaxation are compromised in aging mice even without histopathological alterations (174). cGMP concentration is higher and the response to sildenafil is stronger in the vessels from young mice than in the vessels from old mice. In a mouse model of accelerated vascular aging due to genomic instability, decreased vasodilator function and increased cGMP metabolism in lung tissue were observed but were restored by PDE1 and PDE5 inhibition (175). Thus, cGMP-regulating PDEs may regulate blood pressure, vascular hypertrophy, and possibly vascular senescence. The loss of retinal vascular compliance in aging may contribute to macular degeneration, a leading cause of vision loss in older adults (176). Eyes from older subjects had significant choroidal thinning, and treatment with sildenafil reduced choroidal expansion regardless of the status of age-related macular degeneration.

Aging is associated with progressive loss of cardiovascular and skeletal muscle function and can lead to impaired physical capacity in older adults, likely due to insufficient peripheral O_2 delivery to the exercising muscles (177). A possible underlying mechanism is impaired regulation of blood flow from reduced cGMP signaling in advanced age. Sildenafil increased blood flow to contracting skeletal muscle of older (72 ± 1 years) but not young (23 ± 1 years) male human subjects after submaximal knee-extensor exercise (177). Treatment of older subjects with sildenafil did not blunt α-adrenergic vasoconstriction and improved efficacy of NO-dependent local vasodilator pathways (178). These studies suggest that PDE5 inhibition improves the efficacy of local vasodilator pathways in older subjects during skeletal muscle contractions.

PDE5 IN ALZHEIMER'S DISEASE AND NEURODEGENERATIVE DISORDERS

AD, a neurodegenerative disorder, is the most common form of dementia. AD is characterized by intracellular amyloid plaques consisting of amyloid-β aggregates and extracellular neurofibrillary tangles formed by hyperphosphorylated tau fibrils, which affect proper neuronal functioning (179). Drugs currently used for AD, acetylcholinesterase (AChE) inhibitors or *N*-methyl-D-aspartate (NMDA) antagonists, have very limited efficacy.

The aging brain has lower concentration of cGMP and altered activity of PDE5 and NOS. The potential therapeutic effect of sildenafil as a treatment for cholinergic dysfunction in age-related cognitive decline and AD was first proposed by Devan et al. (180) in 2004. The cGMP-PKG signaling triggered by NO or sildenafil leads to phosphorylation of CREB (**Figure 6**), which can ameliorate altered neuroplasticity and memory deficits in AD (181, 182). Treatment with sildenafil in aging rats significantly improved functional recovery and increased cortical cGMP levels, vascular density, endothelial cell proliferation, synaptogenesis (183), and neurogenesis after focal cerebral ischemia (184). PDE5 is expressed in the human brain and neurons (185) but is not localized in critical brain structures where therapeutic activity is needed (186). However, PDE5 inhibition may produce beneficial effects by alternate mechanisms to combat memory impairment in aged individuals. These mechanisms include inhibition of the increased neuronal apoptosis and dysregulation of neuroplasticity-related molecules [e.g., brain-derived neurotrophic factor and neurotoxic factors, including amyloid-β peptide, associated with the age-related cognitive decline (187)]. Treatment with sildenafil reverted the shift of amyloid precursor protein processing toward Aβ42 production and increased the Aβ42:Aβ40 ratio in aged mice (187).

Inhibitors of PDE2 and PDE5 (Bay 60-7550 and zaprinast, respectively) were studied in 3-, 12-, and 24-month-old rats (188). Bay 60-7550 improved object recognition memory in all three age groups and increased basal constitutive NOS activity in the hippocampus and striatum. Zaprinast improved object memory in 3-month-old rats and elevated NOS activity in all brain regions. In addition, the impaired NO-cGMP-PKG-CREB cascade has been linked to the synaptic deficits—a major hallmark of AD in a mouse model of amyloid deposition. Sildenafil activates PI3-kinase and phosphorylates glycogen synthase kinase-3β (GSK3β), which could increase CREB phosphorylation and ameliorate immediate and long-lasting synaptic function and memory (182). Similar to findings of ischemic protection in heart (31), in vitro studies showed that sildenafil protected neuronal cells from amyloid-β peptide–induced toxicity via opening of mito-K_{ATP} channels (189). The increased NO levels protected against oligomeric forms of tau protein (oTau)-induced impairment of long-term potentiation, a type of synaptic plasticity thought to underlie memory formation through activation of soluble guanylyl cyclase. As outlined in **Figure 6**, pharmacological blockade of cGMP degradation via inhibition of PDE5 rescued oTau-induced reduction of long-term potentiation, rescued memory impairment, and reestablished normal elevation of CREB phosphorylation and cGMP levels (190).

In a recent endophenotype disease module–based methodology, sildenafil was associated with a 69% reduced risk of AD. This conclusion was based on retrospective case-control pharmacoepidemiologic analyses of insurance claim data for 7.23 million individuals (191). It was further demonstrated that sildenafil increased neurite growth and decreased phospho-tau expression in neuron models derived from induced pluripotent stem cells from patients with AD. Clinically, a single 50-mg dose of sildenafil given to patients significantly decreased spontaneous neural activity in the right hippocampus, which is aberrantly increased in the hippocampi and parahippocampal gyri of patients with AD (192). Likewise, a single 50-mg dose of sildenafil given to 12 older-adult patients significantly improved cerebral metabolic rate of oxygen and cerebral blood flow (193).

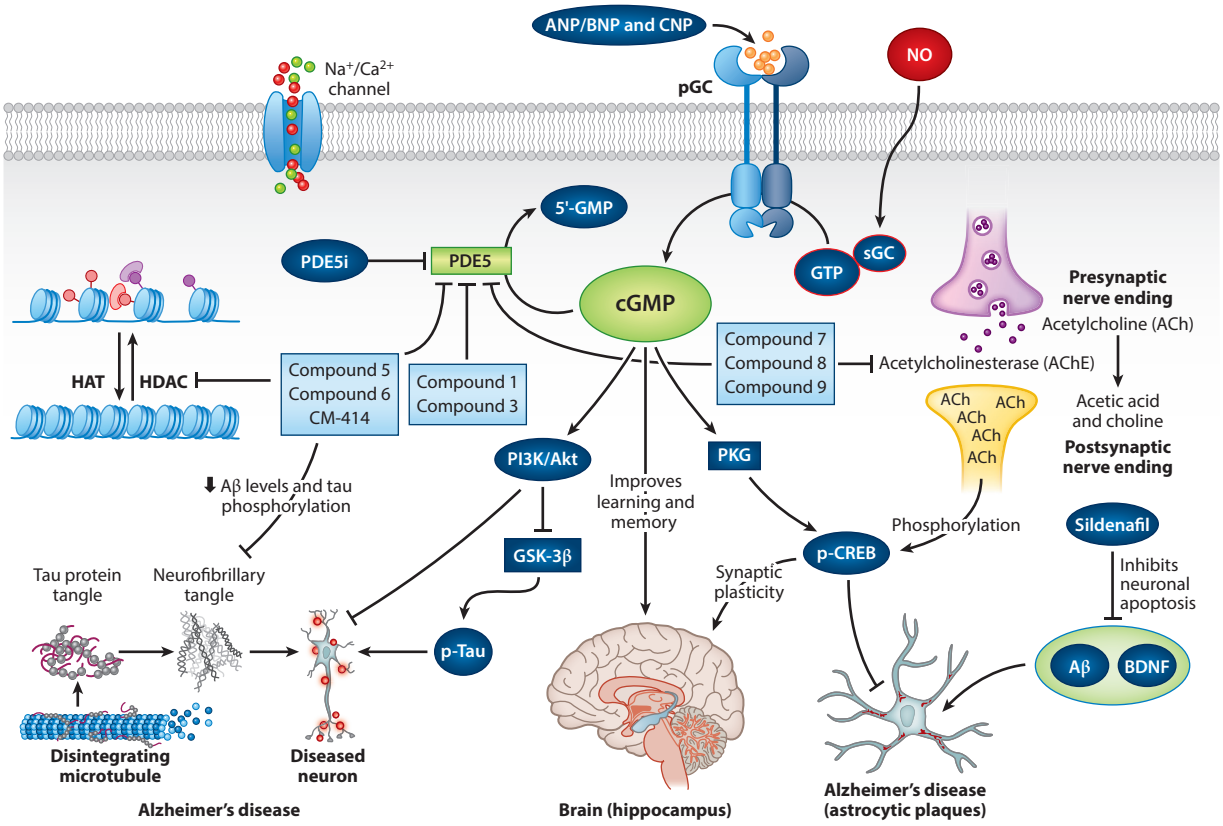

Figure 6

Role of novel inhibitors targeting cGMP-PKG in the treatment of AD. Intracellular amyloid plaques consisting of Aβ aggregates and extracellular neurofibrillary tangles are formed by hyperphosphorylated tau fibrils, which affect neuronal functioning in AD. PDE5 inhibitors may attenuate neuronal apoptosis through inhibition of neuroplasticity-related molecules, including BDNF and Aβ peptide. Novel compounds 1 and 3 are PDE5-specific inhibitors and can readily cross the blood-brain barrier with enhanced efficacy in mouse models of AD. Compound CM-414, a dual inhibitor of HDACs and PDE5, has synergistic therapeutic efficacy in AD models. Compounds 5 and 6 are also dual inhibitors that target both PDE5 and HDACs in AD. Compounds 7–9 are dual inhibitors of AChE and PDE5. Abbreviations: Aβ, amyloid-β; AChE, acetylcholine esterase; AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; cGMP, cyclic guanosine monophosphate; GSK3β, glycogen synthase kinase-3β; GTP, guanosine triphosphate; HDAC, histone deacetylase; pCREB, phosphorylated cAMP-response element binding protein; PDE5, phosphodiesterase 5; PDE5i, PDE5 inhibitor; pGC, particulate guanylate cyclase; PKG, protein kinase G; sGC, soluble guanylate cyclase.

One of the major challenges in treating AD is circumventing the blood-brain barrier and minimizing side effects, since most patients with AD are older adults. Several new PDE5 inhibitors have been developed for treating AD. These include quinoline-based compounds with improved aqueous solubility and excellent potency (in vitro $IC_{50} = 0.056$ nM) (194). As shown in **Figure 1*b***, compound 1, quinoline-based molecules are much more potent inhibitors of PDE5 than are sildenafil, vardenafil, and tadalafil but showed poor water solubility. Compound 3 is naphthyridine based and modified by locking the rotatable bonds of the hydroxymethyl group of the quinoline base to form a ring structure. These alterations increased the water solubility and demonstrated excellent potency and selectivity for PDE5 ($IC_{50} = 0.056$ nM) (182, 195). The conserved Gln817 residue present in the cGMP-binding pocket forms a bidentate hydrogen bond with cGMP. Structural modifications of Gln817 in the Q pocket of the PDE5 catalytic domain

influence the substrate specificity and increase affinity for drugs (196). These compounds readily crossed the blood-brain barrier and showed good in vivo efficacy in a mouse model (197). A first-in-class compound, CM-414, which inhibits both histone deacetylases (HDACs) and PDE5, has a synergistic therapeutic efficacy in AD models (198). Compound 5, designed as a dual inhibitor that can target both PDE5 and HDACs in AD, resembles the 1*H*-pyrazolo[4,3-*d*] pyrimidine skeleton of sildenafil, in addition to the hydroxamic acid moiety, which can inhibit HDAC activity. Compound 5 demonstrated excellent inhibition of PDE5 (IC_{50} = 60 nM) and reasonable inhibition of class I HDACs (HDAC1: IC_{50} = 310 nM; HDAC2: IC_{50} = 490 nM; HDAC3: IC_{50} = 322 nM; HDAC4: IC_{50} = 91 nM) (199). Compound 6 also showed potent inhibition of both PDE5 (IC_{50} = 11 nM) and HDAC6 (IC_{50} = 15 nM). However, in vivo results showed that compound 6 did not result in a significant memory improvement despite decreasing the levels of the AD-related markers amyloid precursor protein and phosphorylated tau protein in Tg2576 neurons (200).

AChE inhibitors are used in clinical practice and are effective against AD. This information led to the development of dual inhibitors that block both AChE and PDE5. As shown in **Figure 1*b***, compounds 7 and 8 are such drugs and are designed by replacing the nitrogen atom of piperazine-2,5-dione (compound 7) and the substituent at the phenyl ring (compound 8) of tadalafil (201). The addition of the ethyl-(1-benzylpiperidin-4-yl) substituent can render AChE inhibitory activity. Both compounds 7 and 8 have good inhibition of AChE (IC_{50} = 36 nM by compound 7; IC_{50} = 32 nM by compound 8) and a modest inhibition of PDE5 (IC_{50} = 153 nM by compound 7; IC_{50} = 1.53 μM by compound 8). Importantly, in vivo studies using compound 7 restored cognitive function and enhanced CREB phosphorylation in scopolamine-induced AD mice. However, compound 7 also had limitation in terms of water solubility and failed to achieve its maximum benefit. To overcome this obstacle, compound 9 was designed to alter the stereo-configuration at positions 6 and 12 of the tadalafil ring. Compound 9 inhibited AChE (IC_{50} = 15 nM) and PDE5 (IC_{50} = 3.23 μM) and improved water solubility (202) (**Figure 6**).

These studies provide encouraging results for the development of new compounds with potential efficacy as PDE5 inhibitors to mitigate pathogenic processes of AD. However, carefully conducted multicenter clinical trials are needed to determine the benefits and safety of PDE5 inhibitors in patients with AD. Moreover, further mechanistic studies are required in order to understand the role of PDE5 in the pathophysiology of AD for future development of novel inhibitors.

PDE5 IN BLADDER DYSFUNCTION AND BENIGN PROSTATIC HYPERPLASIA

Benign prostatic hyperplasia (BPH) is the most common disease in aging males, often comorbid with ED. LUTS-BPH complications from untreated BPH include acute urinary retention, urinary tract infections, and worsening of renal function (203, 204). PDE5 is expressed primarily in the stromal compartment of the prostate, localized mainly in fibromuscular stroma, and upregulated in rat and human BPH (205), making it the main target for PDE5 inhibitors. Treatment with tadalafil reduces proliferation of primary prostate stromal cells and, to a lesser extent, prostate basal epithelial cells (206). The efficacy and safety of tadalafil (5 mg once daily versus placebo over 12 weeks) were evaluated from four multinational, randomized studies of men ≥45 years with LUTS-BPH; analyses were restricted to sexually active men with ED (207). Tadalafil (n = 505) significantly improved total International Prostate Symptom Score (IPSS) versus placebo (n = 521). Because LUTS-BPH and ED are urological disorders that commonly coexist in aging men, tadalafil is more advantageous than other drug options for LUTS-BPH (208). A meta-analysis evaluating the efficacy of PDE5 inhibitors alone or in combination with α-adrenergic receptor blockers for the treatment of LUTS reported significantly improved IPSS and maximum flow

rate values for the group treated with the combination therapy compared with the group treated with the PDE5 inhibitor alone. Thus, α-adrenergic receptor blockers may enhance the efficacy of the PDE5 inhibitors in treating ED and LUTS (209).

THERAPEUTIC PROSPECTS OF PDE5 INHIBITORS IN COVID-19

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can affect pulmonary function and impair oxygenation (210, 211). Binding of the virus to the angiotensin-converting enzyme 2 (ACE2) receptor may cause a renin–angiotensin system imbalance with excessive activation of the ACE–angiotensin II–angiotensin type 1 receptor pathway, which is in line with a reduction in the ACE2–angiotensin-(1–7)–MAS receptor pathway (212). In addition, SARS-CoV-2 triggers innate immune responses, including the activation of monocytes and complement cascade, which is responsible for local release of proinflammatory cytokines and aggravation of endothelial injury and microvascular thrombosis, termed immunothrombosis (213). The viral infection can induce the activation of macrophages and immunological reactions that result in excessive release of proinflammatory cytokines and chemokines (cytokine storm) (214). These inflammatory mediators injure epithelial cells in the lungs and via the circulation can damage other organs (215).

Elevated levels of endothelial angiotensin II also stimulate the generation of reactive oxygen species (ROS), which can breakdown NO (216). There may also be a disequilibrium in NO generation from iNOS and eNOS. Production of NO by eNOS is associated mostly with tissue protection, while excessive NO formation from iNOS can trigger a proinflammatory cascade that usually results from oxidative stress (217). Depletion of NO leads to vasoconstriction, with progressive ventilation/perfusion (V/Q) mismatch (211). Because PDE5 is expressed predominantly in the lungs, the use of PDE5 inhibitors as a potential treatment for COVID-19 has been proposed (70, 71, 113). In this context, the NO-cGMP-PKG pathway triggered by PDE5 inhibitors could be an attractive possibility for the treatment of patients with COVID-19. eNOS activation is mediated partly by AMPK (217), which quells inflammation through its inhibitory effects on iNOS. The administration of sildenafil in patients with cerebellar demyelination resulted in downregulation of inactive AMPK and iNOS (218) and inflammation associated with COVID-19 (**Figure 7**). Enhancing the AMPK-eNOS-NO-cGMP pathway can potentially counteract thromboembolism in patients with type 2 diabetes (219). PDE5 inhibitors may improve the prognosis of pulmonary inflammation caused by SARS-CoV-2 infection (220). Isidori et al. (217) designed a clinical trial, the DEDALO project (silDEnafil administration in DiAbetic and dysmetaboLic patients with COVID-19), to assess whether PDE5 inhibitors could help manage COVID-19 by (*a*) counteracting the angiotensin-II-mediated downregulation of angiotensin II receptor type 1 (AT-1); (*b*) acting on monocyte switching, thereby reducing proinflammatory cytokines, interstitial infiltration, and the vessel damage responsible for alveolar hemorrhage and necrosis; and (*c*) inhibiting the transition of endothelial and smooth muscle cells to mesenchymal cells in the pulmonary artery, preventing clotting and thrombotic complications. A recent meta-analysis suggested that sildenafil inhibited apoptosis of lung epithelial cells and combination treatment of sildenafil with epoprostenol (a prostaglandin) and bosentan (an endothelin receptor antagonist) had anti-inflammatory effects and reduced pulmonary artery blood pressure, lung edema, and vascular remodeling (221). Thus, it appears that PDE5 inhibitors could potentially play a role as adjuncts in the mitigation of COVID-19 complications by modulating the NO-cGMP-PKG pathway.

POTENTIAL ADVERSE EFFECTS OF PDE5 INHIBITORS

The most well-known contraindication of PDE5 inhibitors is with organic nitrates (222), which are used as vasodilators for the treatment of angina and HF. When organic nitrates are taken

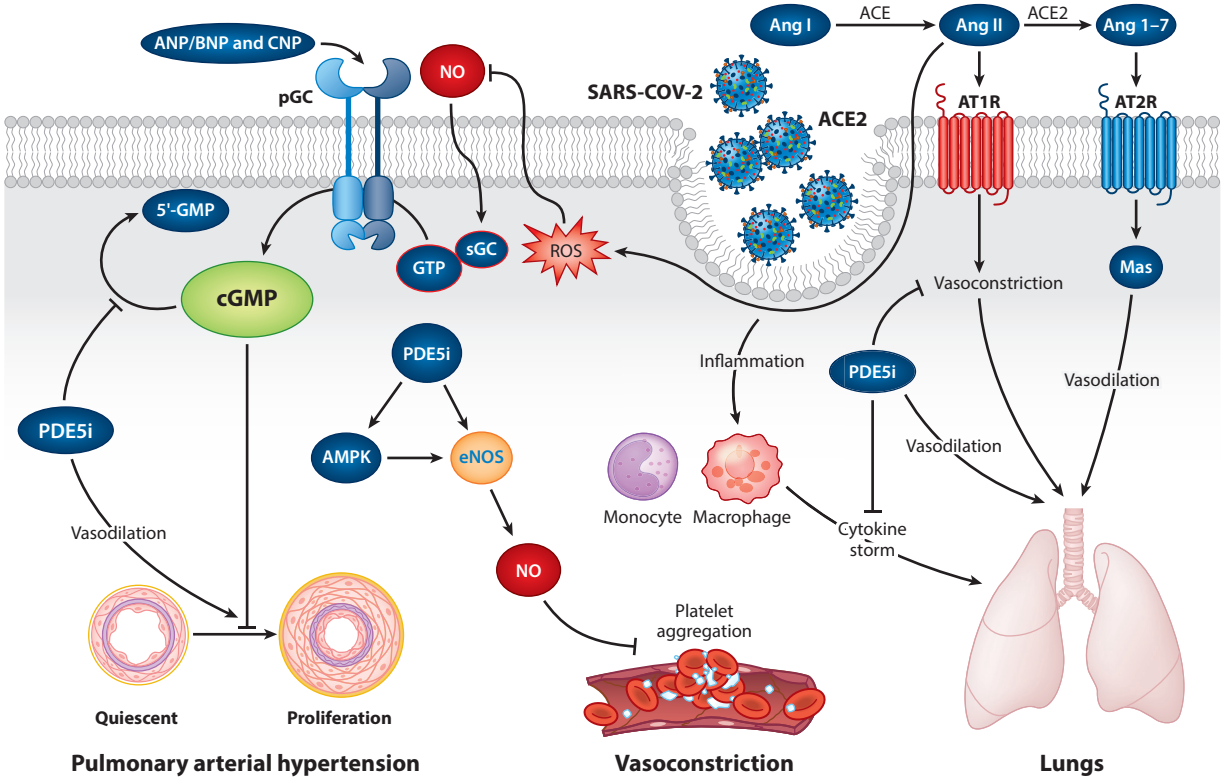

Figure 7

PDE5 inhibition in the treatment of COVID-19 and pulmonary hypertension. The binding of the SARS-CoV-2 virus with its ACE2 receptor leads to excessive activation of the ACE–angiotensin II–angiotensin type 1 receptor pathway. Angiotensin II stimulates ROS with potential depletion of NO, which triggers proinflammatory cascade for vasoconstriction. The NO-cGMP-PKG pathway activated by treatment with PDE5 inhibitors restores NO through AMPK-mediated eNOS activation, thereby attenuating inflammation and inhibition of platelet aggregation. PDE5 inhibitors also significantly increase pulmonary vasorelaxation and attenuate pulmonary arterial hypertension. Abbreviations: ACE2, angiotensin-converting enzyme 2; AMPK, AMP-activated protein kinase; Ang I/II, angiotensin I/II; AT1R/AT2R, angiotensin II receptor type 1/2 receptor; cGMP, cyclic guanosine monophosphate; eNOS, endothelial nitric oxide synthase; GTP, guanosine triphosphate; NO, nitric oxide; PDE5, phosphodiesterase 5; pGC, particulate guanylate cyclase; PKG, protein kinase G; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; sGC, soluble guanylate cyclase.

together with a PDE5 inhibitor, systemic blood pressure can drop excessively, with resultant symptoms/illness and injury. Nevertheless, a recent large retrospective observational study reported no significant difference in cardiovascular outcomes in patients coadministered with PDE5 inhibitor and nitrate versus nitrate only (223). Also, taking tadalafil together with antihypertensive medications did not increase the risk of hypotension-related adverse events or major adverse cardiovascular events (224). PDE5 inhibitors weakly inhibit PDE6, which is located in rod and cone photoreceptors and may cause mild and transient visual symptoms (225). Because PDE5 is present in choroidal and retinal vessels, its inhibition increases choroidal blood flow and vasodilation in the retinal vasculature. The ophthalmologic side effects may also include nonarteritic ischemic optic neuropathy (NAION), chorioretinopathy, glaucoma, and optic atrophy (226). A randomized, double-blind, placebo-controlled clinical trial in 20 healthy young men 20–40-years-old showed that a single oral dose of 100 mg of sildenafil caused transient changes of

outer and inner retinal function, as detected by electroretinogram and psychophysical methods. However, the acute effects were fully reversible within 24 hours (227). Also, a recent meta-analysis revealed that PDE5 inhibitors were not associated with NAION and had a relatively acceptable ocular safety profile (228).

CONCLUDING REMARKS

Dysregulation of NO-cGMP-PKG signaling plays a critical role in a variety of diseases, including urological disorders, cardiovascular disorders, cancer, aging-related complications, and genetic disorders, such as DMD. PDE5 inhibitors have played an important role in improving the quality of life for men (being first-line therapy in ED) and in treating PAH and LUTS. PDE5 is expressed in many tissues, which implies the potential for new indications for PDE5 inhibitors. Experimental data, and to a lesser extent clinical studies, suggest that PDE5 inhibitors are cardioprotective in the setting of I/R injury, HF, diabetes, and cancer. There is also growing evidence that PDE5 inhibitors have potential to treat aging-related diseases, including AD, and as adjunct therapy to improve COVID-19 outcome by modulating the NO-cGMP-PDE5 axis. In consideration of the established safety record of PDE5 inhibitors, repurposing these drugs may offer an attractive option for future treatments of many human diseases.

SUMMARY POINTS

1. Phosphodiesterase 5 (PDE5) is involved in the pathophysiology of several diseases due to dysregulated nitric oxide–cGMP–protein kinase G signaling.
2. Because of the widespread expression of PDE5 in numerous tissues and organs, it is a target for potential treatment of various diseases.
3. PDE5 inhibitors, including sildenafil, are clinically used to manage erectile dysfunction, pulmonary arterial hypertension, and lower urinary tract symptoms of benign prostatic hyperplasia.
4. Experimental results and some clinical data suggest the potential for PDE5 inhibitors in the treatment of diseases that include cardiovascular disorders, Alzheimer's disease, and cancer, among others.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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