

# Expert Opinion

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## Chronic sildenafil in men with diabetes and erectile dysfunction

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Erectile dysfunction frequently represents a neurovascular complication of diabetes mellitus, and it has been calculated that almost 50% of diabetic men will have erectile dysfunction within 6 years after diagnosis. Penile endothelial and smooth muscle cell dysfunction are due to molecular pathway abnormalities (i.e., activation of PKC, increased oxidative stress and overproduction of advanced-glycosylation end products). The response rate to oral drug therapies, such as sildenafil, is lower than in most other groups. Because therapeutic alternatives (i.e., intracavernous injections with vasoactive agents) are not curative, clinical trials aimed to demonstrate rehabilitative effects with daily phosphodiesterase type-5 inhibitors are ongoing. If this approach proves successful, it will determine many advantages over the intracavernosal treatment and potentially induce sexual rehabilitation.

**Keywords:** diabetes, endothelial dysfunction, erectile dysfunction, phosphodiesterase type-5, rehabilitation

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### 1. Introduction

Erectile dysfunction (ED) is defined as the consistent or recurrent inability of a man to attain and/or maintain a penile erection sufficient for sexual activity [1]. Male ED may constitute the first manifestation of important systemic or relational pathologies and it is considered a possible marker of clinically undiagnosed disease, thus representing the 'tip of the iceberg' of a systemic vascular disorder [2]. It is common (prevalence of 50%) in men with diabetes mellitus (DM) and is often associated with vascular disease, such as coronary and peripheral atherosclerosis, and an increased risk of future cardiovascular events similar to that of men with coronary artery disease (CAD) [3,4]. Therefore, regarding the risk of cardiovascular events, DM can be considered equivalent to established CAD [5]. The incidence of DM is increasing at an alarming rate, and diabetic men already make up a quarter of the men in the ED population. As already outlined, ED may occur in up to 50% of men with DM [6] and the rate of reported sexual dysfunction is only slightly lower in diabetic women [7]. ED onset occurs at an earlier age in diabetic men, being mostly noted within 10 years after the onset of DM; in contrast, it may represent the first sign of undiagnosed diabetes in as much as 10% of patients [8]. Furthermore, ED seems to occur at an earlier age in Type 1 than in Type 2 diabetic patients, although it probably occurs with equal frequency in both types [9]. Ageing, cigarette smoking and the presence of diabetic complications (peripheral neuropathy, vascular disease, retinopathy and nephropathy) show a significant relationship with the occurrence of ED in both Type 1 and 2 diabetes [10]. A large body of evidence has accumulated to suggest that the impairment of vascular endothelial function is an initial step towards the development of atherosclerosis. Endothelial function is impaired in patients with overt atherosclerotic disease as well as in those at increased cardiovascular risk, and it is often present in men with DM and without clinically evident atherosclerotic

diseases [11,12]. In addition to blunted endothelium-dependent and -independent responses, diabetic patients are reported to have a reduced response to vasoactive agents so it is reasonable to assume that treatments with drugs which improve endothelial function (i.e., metformin or sartanes) may be somehow beneficial [13]. The recent discovery that chronic administration of phosphodiesterase type-5 inhibitors (PDE5-i) may improve erectile and endothelial response in men previously unresponding to on-demand regimes opens a new scenario in the treatment of men with ED and comorbidities [14].

PDE5-i is a group of (on-demand) drugs licensed for the treatment of ED and appear to offer advantages over past therapies in terms of ease of administration and patient preference, and they are now widely advocated as first-line therapy. Sildenafil was the first PDE5-i to be released (1998 in European Union) and has been studied extensively (> 1000 papers have appeared on MEDLINE). More recently, two other agents, vardenafil and tadalafil, have been introduced. Although the various classes of PDE5-i differ with respect to selectivity and pharmacokinetic profiles, their efficacy and safety profiles are almost comparable in broad populations of men with ED. Sildenafil is a specific PDE5-i initially developed for cardiovascular use, but then approved for the treatment of ED as it facilitates erection by increasing nitric oxide (NO)-determined relaxation through the inhibition of cGMP breakdown in endothelial cells [15-17]. However, because PDE5 is widely expressed in the vasculature [18], sildenafil enhances NO-mediated responses in other vascular beds. Efficacy of PDE5-i is reported to be lower in diabetic patients with ED compared with those without diabetes [19], akin to the effectiveness of other groups of drugs known to improve endothelial function [20], not only because of impaired NO production, but also because of decreased availability of precursors. It has been suggested that chronic sildenafil administration may regulate the transduction pathway leading to the activation of endothelial-NO synthases (eNOS) with no effect on NO bioavailability or on the cGMP pathway, thereby eliminating a possible concern for tachyphylaxis. [21] Recently, it has been demonstrated in humans that chronic administration of PDE5-i may improve endothelial function long-term [22].

## 2. Pathophysiology of diabetic erectile dysfunction

The role of neurogenic and vascular factors in the aetiology of diabetic ED is well recognised. Historically, it was considered that diabetes may somehow lead to gonadal dysfunction with associated endocrine abnormalities, and that this contributed to the pathophysiology of the condition. Attention is increasingly being focused on the role of the vascular endothelium and the control of smooth muscle tone within the penis. Each of the pathological effects of DM in tissues, such as the alterations in small arteries and arterioles, neurological demyelination and sinus smooth-muscle deterioration, has been indicated as the possible aetiological factors associated with

ED. Hypertension is a frequent comorbidity, being present in 40 – 60% of diabetics in the literature [23]. Other frequent comorbidities are long-term cigarette smoking, depression, obesity and hyperlipidaemia, as well as many commonly prescribed pharmacological agents with antierecile properties.

### 2.1 Endothelial and smooth muscle factors

In the penile corpora cavernosa (CC), the endothelium lining the lacunar spaces is important in controlling corporal smooth muscle tone. NO, constrictor prostanoid and endothelins are all produced by the endothelium and act directly on the smooth muscle cells [24]. It has been shown that in the diabetic penis there is a decreased amount of both neuronal NOS and eNOS and, in turn, decreased local production of NO by these isoenzymes. Recent evidence has demonstrated that arginase, which competes for the same substrate of NOS (i.e., L-arginine), is expressed in human CC and that its inhibition facilitates NO-dependent relaxation of penile CC smooth muscle. Along with this knowledge, it has been hypothesised that the biology of arginase is altered in diabetic penile tissue contributing to reduced NOS in the CC [25]. In fact, the evaluation of the arginase gene expression, protein levels and enzyme activity showed that all parameters of arginase-II, but not arginase-I, are increased in diabetic tissue and that the inhibition of arginase activity reversed the reduction in NOS in this tissue [26]. These findings support the possibility that an alternative mechanism for impaired NO-dependent penile erection is present in diabetic ED, expanding the knowledge of NO regulation in the penis and fostering new therapeutic possibilities [27]. Diabetes is associated with increased production of advanced glycosylated end products (AGEs) [28]. AGEs may influence several ion channels and receptors functioning at the molecular level and also quench NO directly, thus obliterating its effects. Furthermore, it has been reported that in diabetic patients there is an impaired autonomic nerve-mediated and endothelium-dependent relaxation of corporal smooth muscle, whereas autonomic nerve-mediated contraction is maintained [29]. The neurogenic dysfunction results in the inability of the corpora to fully relax following sexual stimuli and, along with impaired vasodilatation, further contributes to the severity of ED [30]. The longer the duration of diabetes with a modest diabetes control, the less pronounced is the neurogenic relaxation; no differences were observed between diabetics treated and not treated with insulin, nor were there differences in diabetic patients when controlled for hypertension and cigarette smoking. Neurogenic relaxation may be normal in diabetic patients when induced by endothelium-independent vasodilators (i.e., sodium nitroprusside and papaverine) [31]. Sullivan *et al.* demonstrated that in the rabbit CC there is a significant increase in endothelin (ET)-B receptor-binding sites 2 months after the induction of DM by alloxan [32]. Because ET-B receptors can be found on endothelial cells, where they stimulate NO production, the authors suggested that this could represent another compensatory pathway involved in diabetic ED pathophysiology [33]. Indeed, plasma concentration of ET-1

are higher in non-diabetic and diabetic men with ED when compared with controls, and they are more elevated in diabetic than in non-diabetic patients [34]. However, other investigators cannot find any difference in ET-1 activity in DM [35]. Another process that is accelerated in diabetics, is the change in collagen amount and quality within the CC [36]. At the molecular level, changes in apoptosis and TGF-1 expression are also associated with diabetic penile changes [37] and it has been suggested that hypoxia-induced platelet-derived growth factor overexpression may influence vasoconstriction as well as smooth muscle cell relaxation [38]. Most of these changes may affect the mechanisms involved in vasodilatation and smooth muscle cell relaxation.

## 2.2 Vascular factors

The penis is a vascular organ. Factors that impede the blood flow into the penile helicine vessels and sinusoidal spaces will lead to ED. DM is associated both with atherosclerosis in large arteries (which appears more frequently and at an earlier age than in non-diabetics) and with microangiopathy, characterised by increased thickening of the capillary basement membrane. It has been reported that diabetic macrovascular complications are related to age, whereas microvascular complications are affected by the duration of diabetes and degree of glycemic control. Arteriographic studies have demonstrated that stenosis of the internal pudendal artery is more common in impotent than potent diabetics, and duplex ultrasound scanning of the penile arteries has shown that, in impotent men, DM is associated with a smaller penile artery diameter and lower peak flow velocities following injection of an intracorporal vasoactive agent [39]. In addition, morphological studies of diabetic tissue have demonstrated ultrastructural changes within small penile vessels, including endothelial proliferation, subintimal fibrosis and endarteritis obliterans. DM is also associated with other conditions that might cause vascular problems. For example, there is an increased risk of hypercholesterolaemia and hypercoagulability [40]. Hypercholesterolaemia is a risk factor for ED in its own right, and leads to an increased risk of atherosclerosis; at the cellular level, it can lead to increased contractility and impaired endothelium-dependent relaxation of the CC smooth muscle. The hypercoagulability associated with diabetes is secondary to an increase in coagulation factors such as Factor IX (Von Willebrand factor) and tissue plasminogen activator inhibitor, which in turn may facilitate subsequent vessel thrombosis and reduced vascular inflow, thus predisposing patients to tissutal scarring and plaque formation [41]. Clinical studies have clearly demonstrated a close correlation between ED and other manifestations of diabetic vascular disease, such as retinopathy and claudicatio intermittens.

## 2.3 Neurogenic factors

The development of neuropathy in men with DM might have a role in the appearance of ED. Neuropathy initially affects small unmyelinated fibres and leads to functional abnormalities

in a number of organ systems. For example, there may be postural hypotension and disorders of gastrointestinal tract motility as well as alterations of thermal sensation and abnormalities of sweating. In the later stages of the disease, larger myelinated fibres are also affected, with the longest fibres usually being affected first. This produces the classical 'glove and stocking' distribution of the peripheral neuropathy. Morphological evidences have demonstrated changes in the innervation in diabetic tissues as well as in the dorsal nerve of diabetic rats, with a reduction in the size of myelinated nerve fibres and accumulation of glycogen in axons and lipid droplets in Schwann cells, but no changes within the cavernous nerve [42]. Human studies provided conflicting evidence: although light microscopy and electron microscopy suggested changes in the nerves of the CC in some patients, these changes have not been demonstrated in other groups [43]. Both in human and animal models, the bulk of evidence seems to suggest a depletion of neurotransmitters at the cellular level. Early studies have demonstrated a marked reduction in vasoactive intestinal polypeptide, acetylcholinesterase and NOS immunoreactivity [44]. In addition, it has been demonstrated that in the erectile tissue of men with DM there is a reduction in noradrenaline levels, suggesting that neuropathy may affect sympathetic and parasympathetic nerve fibre outflow. Usually, sympathetic reactivity is enhanced in diabetic penile tissues as compared with controls, thus impairing the balance between endothelial relaxing and contracting factors [45].

## 2.4 Endocrine factors

Both erectile function and sexual physiology are reliant on a normal endocrine milieu, which is provided by a normally functioning pituitary, hypothalamus, adrenal gland and testis. The pathophysiological importance of abnormalities in these axes with respect to diabetic ED is controversial. Studies performed in diabetic rats have demonstrated both a reduced serum testosterone (T) and size of androgen-sensitive accessory reproductive organs. Hypogonadism may play a significant role in the pathophysiology of ED. A threshold level of T may be necessary for normal erectile function and T replacement therapy is indicated in hypogonadal patients with ED. Monotherapy with T for ED may be of limited effectiveness and may be most promising in young patients with hypogonadism and without vascular risk factors for ED. A number of laboratory and human studies have shown the combination of T and other ED treatments is effective in treating the symptoms of ED in patients for whom treatment failed with T or sildenafil alone [46]. Studies performed in humans have found that hypogonadism is common in DM and that treatment with T is beneficial for penile erection [47]. For example, it has been shown that in men with DM and primary organic ED there are lower serum-free T levels when compared with both normal and diabetic men with primary psychogenic ED [48]. This study also showed improvements in sexual function following therapy with parenteral T. However, in a recent report based on 1000 consultations for ED, it has been found that there is

no difference in hypothalamus–pituitary–testicular axis alterations in diabetic males when compared with men with other chronic illnesses, thus suggesting that hypogonadism may be present in few men with diabetic ED, primarily due to a gonadal abnormality [49]. A possible explanation for this phenomenon is the important contribution to sexual hormone alterations due to changes in circulating sex-hormone binding globulin (SHBG) levels occurring in chronic diseases, because total T concentrations are determined to a large extent by circulating SHBG concentrations. In the blood of normal men, 44% of total T is bound to SHBG, 2% is unbound (free T) and 54% circulates bound to albumin and other proteins. Because albumin-bound T has 1000-times lower affinity than SHBG, it can freely disassociate in capillaries. Virtually all the non-SHBG-bound T (also called bioavailable T) is, therefore, available for tissue uptake. Circulating SHBG concentrations are also dependent on a number of factors, the most important association being with obesity. SHBG levels decrease in obesity and increase with ageing. Men with Type 2 DM have even lower SHBG levels compared with age- and body-mass index-matched non-diabetics and this may determine an underestimation of hypogonadism. A complete assessment of hypogonadism should, therefore, include measurement of free T levels, which in most studies led to estimate a true incidence of 30 – 40% [50]. Thus, the poor responsiveness to PDE5-i often observed in diabetic patients might be due to the frequently associated T deficiency. Interestingly, patients affected by diabetes-associated hypogonadism and non-diabetic hypogonadal subjects share the same symptoms and signs, including depression, reduced libido and alteration in penile vascular parameters [51]. Because previous clinical studies indicated that T supplementation ameliorated PDE5-i responsiveness in hypogonadal patients [52], further clinical trials will help to establish whether treating hypogonadism might restore sildenafil responsiveness even in diabetic patients, as reported in animal models [53]. If this is the case, diabetes-associated hypogonadism and its related clinical consequences, including sexual dysfunction and poor responsiveness to standard therapeutic regimens, will have a novel therapeutic option available in T substitution.

### 2.5 Psychogenic factors

Although the majority of men with DM often display organic ED, a number of patients may have additional psychological factors [54]. Epidemiological studies tend to underestimate the true dimension of the problem because of the embarrassment or stigma that is associated with ED. Men with ED may experience diminished self-image and self-esteem, anxiety and fears of rejection, and even depression as psychogenic factors. Some studies have shown that up to 30% of diabetic men with ED have significant emotional components that contribute to their sexual dissatisfaction.

It is easy to understand that in the era of orally active drugs, the majority of ageing patients who complain of sexual disturbances will expect a treatment in order to restore their

erectons. This will lead to an increasing number of new prescriptions of PDE5-i, with a relatively high social cost and impact on quality of life. However, a high dropout rate is expected to occur as is true with all successful therapies. Thus, men and their partners need to be educated and/or counselled about how to achieve satisfactory coital experiences. The treatment should go beyond the penis itself and encompass the relationship and the partner.

### 3. Pharmacology and pharmacokinetics of sildenafil

PDEs are intracellular enzymes that hydrolyse cAMP and cGMP to their respective linear 5'-nucleoside monophosphate. By controlling cAMP and cGMP concentrations, PDEs play a critical role in a wide range of physiological and pathological processes, and are thus potential targets for drug interventions. The small sizes and simple structure of their substrates suggest the likelihood of modulating their activities with small molecules that are structurally analogous to these substrates [55]. As such, in recent years, hundreds of PDE-inhibitors have been tested in numerous clinical trials as, for example, anti-inflammatory, antiasthmatic, antithrombotic or antidepressant agents. The main obstacle in bringing the rest of the PDE-inhibitors to the market is the unwanted side effects that these compounds all tend to cause. It has now become clear that the underlying mechanism is the multiplicity of PDE families and their isoforms that are widely distributed, yet often overlapping, in various tissues, such as vascular smooth muscle, testis, heart, epithelial smooth muscle, brain, liver, neutrophils, lung, penis, rod and cone photoreceptor cells and T lymphocytes and so on. Moreover, the fact that different PDEs catalyse the same substrate underscores the likelihood of the test compound's crossreactivity with different PDEs, thus exacerbating the undesirable side effects [56,57].

The best-characterised physiological role of PDE5 is the termination of the cGMP-signalling pathway in smooth muscle, be it vascular, cavernous or intestinal. Sexual stimulation triggers the release of NO from nerve endings. NO diffuses into cavernous smooth muscle cells and activates guanylyl-cyclase, which then catalyses the conversion of GTP to cGMP. Elevated levels of cGMP activate protein kinase G, which then phosphorylates gap junctions, K<sup>+</sup> channels and Ca<sup>2+</sup> channels. Phosphorylation of the K<sup>+</sup> and Ca<sup>2+</sup> channels leads to an increase in potassium efflux and reduction of calcium influx, respectively. When the cytoplasmic calcium concentration falls < 500 nM, calcium dissociates from calmodulin, which in turn dissociates from the myosin light-chain kinase, thus inactivating it. With its kinases being inactivated and its phosphates being removed by phosphatase, the myosin light chain thus becomes dephosphorylated. Dephosphorylated myosin light chain inhibits the binding of the myosin head to actin, resulting in smooth muscle relaxation. Relaxed cavernous smooth muscle allows the entry of blood into the cavernous spaces, thus expanding the penis. To

**Table 1. Principal drugs used by diabetic patients and potential interactions with sildenafil.**

Class of drug	Interaction with sildenafil	Effects
Nitric oxide donors	Yes	Severe hypotension
Angiotensin-converting enzyme inhibitors/sartanes	No	-
$\alpha$ -Blockers	Yes	Mild postural hypotension
$\beta$ -Blockers	No	-
Diuretics/anticoagulants	No	-
Statins	Yes	Possible rhabdomyolysis
<b>Other substances of common use</b>		
Rifampicin	Yes	Reduces sildenafil half-life
Erithromicin	Yes	Increases sildenafil half-life
Antiretroviral drugs	Yes	Increase sildenafil half-life
Azole antifungals	Yes	Increase sildenafil half-life
Cimetidine	Yes	Reduces sildenafil clearance
Grapefruit Juice	Yes	Increases sildenafil half-life

return the penis to the flaccid state, cGMP is principally degraded by PDE5, despite the co-presence of several other cGMP-hydrolysing PDEs. In addition, the naturally flaccid state of the penis is likely maintained with the participation of PDE5 [58].

Sildenafil citrate (Viagra<sup>®</sup>, Pfizer) is the first effective oral agent in the management of ED having a revolutionary impact on management of ED from its introduction in 1998. It acts by blocking cGMP degradation via PDE5 inhibition and, in turn, enhances the effect of sexually induced NO in causing penile smooth muscle relaxation and erection. The most prominent effect of the drug is exerted on PDE5, the most abundant enzyme identified in the human CC. Sexual stimulation is mandatory for sildenafil to produce its beneficial pharmacological effects on erectile function in impotent men, therefore, it is generally considered to be a peripheral conditioner [59]. Differences in pharmacokinetic properties among the PDE5-i include the fact that sildenafil and vardenafil are short-acting agents (half-life: ~ 4 h), whereas tadalafil (half-life: 17.5 h) is a long-acting one, thus allowing the patient more flexibility in planning sexual activity. In healthy men with ED, onset of activity with sildenafil may be seen as early as 11 min post dose [60]. In one study, within 14 and 20 min of dosing, 35 and 51% of patients treated with sildenafil, respectively, versus 22 and 30% of patients receiving placebo, respectively, had one or more erections leading to successful intercourse. The median time after sildenafil dosing that led to erection resulting in successful intercourse was 36 min (versus 141 min for placebo) [61]. Sildenafil absorption may need 30 – 90 min before an optimal effect on erection occurs in the absence of concomitant meal assumption. In fact, the presence of high-fat food may delay absorption of sildenafil [62]. Moreover, it is reasonable to postulate that in the absence of complete PDE5 inhibition, partial dephosphorylation of cGMP will decrease

the erectile response. Therefore, men of any age who do not initially respond to sildenafil should be encouraged to try up to a maximum dose of 100 mg on 6 – 8 different occasions before a lack of response is considered. Sildenafil is rapidly absorbed after oral administration and the half-lives of these drugs are of 4 – 5 h with a bioavailability of 40%. It is metabolised by the CYP enzyme system located in the liver, being substrates of CYP3A4 and metabolised to a des-methyl derivative. Metabolites of sildenafil (and vardenafil) have a minor contribution to the biological activity of these drugs (of ~ 7 – 20%), whereas those of tadalafil are devoid of biological activity. All drugs and their metabolites are excreted in the faeces [63]. There are a number of interactions between sildenafil and drugs metabolised by the CYP3A4 pathway (Table 1). Being potent inhibitors of CYP3A4, these drugs may interact with other compounds that act as substrates/inducers of this cytochrome system, such as the HIV-1 protease inhibitors (e.g., ritonavir, indinavir, etc.) [64] and the azole antifungals (erythromycin, cymetidine, etc.), which lead to an increase in the systemic exposure of the PDE5-i of 2- to 16-fold, thus determining symptomatic hypotension [65]. For example, ritonavir increases the systemic exposure (AUC) of sildenafil 11-fold, and caution should be exercised when prescribing the two drugs together. Because of its inhibiting action on intestinal CYP3A4, grapefruit juice also increases sildenafil bioavailability and tends to delay sildenafil clearance. Although patients will not usually be endangered by concomitant use of grapefruit juice, it seems advisable to avoid this combination. By contrast, CYP3A4 inducers, such as rifampicin, reduce the circulating levels of PDE5-i [66]. Furthermore, as vasodilators, these drugs potentiate the vasodilator/hypotensive effects of NO donors, such as the organic nitrates (nitroglycerin, isosorbite dinitrate, isosorbite mononitrate) and the two drug classes association is absolutely contraindicated [67]. Acute

**Table 2. Stratification of cardiovascular risk factors according to 'The Second Princeton Consensus Conference' and indications to sexual activity according to low-intermediate or high risk (adapted from [115]).**

Low Risk	Intermediate/indeterminate risk	High risk
Asymptomatic, < 3 risk factors*	Asymptomatic, ≥ 3 risk factors	Unstable or refractory angina
Controlled hypertension	Moderate, stable angina pectoris	Uncontrolled hypertension
Mild, stable angina pectoris	MI > 2 weeks but < 6 weeks	CHF (NYHA class III, IV)
Post revascularisation and without significant residual ischaemia	LVD/CHF (NYHA class II)	Recent MI (< 2 weeks)
Post MI (> 6 – 8 weeks) but asymptomatic and without ETT-induced ischaemia	Noncardiac atherosclerotic sequelae	High-risk arrhythmias
Mild valvular disease		Obstructive hypertrophic cardiomyopathies
LVD (NYHA class I)		Moderate-to-severe valve disease

\*Age, hypertension, diabetes mellitus, cigarette smoking, dyslipidemia, sedentary lifestyle, family history of premature coronary artery disease.

CHF: Congestive heart failure; ETT: Exercise tolerance test; LVD: Left ventricular dysfunction; MI: Myocardial infarction; NYHA: New York Heart Association.

postural hypotensive symptoms have seldom been reported when sildenafil is administered with  $\alpha$ -adrenergic blockers (such as doxazosin). It is prudent to start the PDE5-i at a low dose (25 mg) in patients who have already adjusted to a stable dose of an  $\alpha$ -blocker [68], but doses > 50 mg can be given > 4 h following dosing of sildenafil.

Finally, it is noteworthy to remember that statins have cholesterol-independent effects, including an increased vascular NO activity, and are commonly used by patients with diabetes, cardiovascular disease and ED. Such patients should take advantage of the favourable effects of such combination on vascular sensitivity [69], but must be warned because of potential drug interactions of both drugs that are highly protein bound and are metabolised by the liver via the CYP3A4 pathway. It is possible that this competition causes a rise in the statin levels, increasing the risk for rhabdomyolysis [70].

Adverse events or side effects related to vasodilatation are the most commonly observed post sildenafil ingestion. Consistent with its mechanism of action, headaches (16%), flushing (10%), nasal congestion (4%) and gastroesophageal reflux (7%) occur ~ 1 h after intake, whereas visual disturbances (blue-green hues, hypersensitivity to light) seldom occur (3%) and are more frequent post sildenafil 100 mg administration [71]. Side effects are generally reversible and tend to diminish during continued treatment. Overall, discontinuation of sildenafil because of adverse events of all types in placebo-controlled studies was comparable in the sildenafil (2.5%) and placebo (2.3%) groups. Long-term cardiovascular safety and, in particular, the potential for sildenafil to cause death and/or serious adverse events in men with ischaemic heart disease have been of primary concern [72], but postmarketing data report that the rate of death and/or serious adverse events reported to FDA is not inconsistent with the baseline mortality rate in men of this age and health status [73]. Placebo-controlled and open-label Phase II – III trials, including men with ischaemic heart disease, did not show an increase in myocardial infarction (MI) or serious cardiovascular events in patients treated with sildenafil versus placebo. None of the serious cardiovascular events reported in these trials were

considered treatment-related by the investigators. There is a small, but finite, increased risk of developing ischaemia or MI with sexual activity exercise in men with known or probable CAD [74]. Therefore, before prescribing sildenafil or any existing or future treatment for ED to patients with DM, cardiac disease or multiple cardiovascular risk factors, physicians should discuss the potential cardiac risk of sexual activity and perform a complete medical assessment, including an exercise stress-test if appropriate [75]. The second Princeton Consensus Conference has highlighted the relation between ED and cardiovascular disease (CVD), stratifying ED patients in three risk classes [76]: low-risk patients could immediately initiate a treatment for ED without further cardiac evaluation; patients with intermediate or indeterminate risk should be clinically evaluated and reclassified as high or low risk patients; and high-risk patients should defer resumption of sexual activity until cardiological assessment and specific treatment has been defined (Table 2).

Priapism and prolonged erection are rare adverse events that have been reported with this class of drugs [77]. In addition, most classes of PDE5-i inhibit the photoreceptor PDE6 isoform rather well, similarly to the PDE family to which they were targeted (i.e., PDE5 in this case). In intact rod outer segments, high PDE6 concentrations are present, and the binding of inhibitors to the  $\gamma$ -subunit to the active site and calcium feedback mechanisms attenuate the effectiveness of PDE inhibitors, leading to inhibition of PDE6, disruption of the cGMP signalling pathway during visual transduction and some transitory visual side effects of this entire class of drugs [78].

Visual side effects (for example, light sensitivity, colour vision abnormalities) associated with sildenafil use are well documented and are supposed to be related to the fact that it appears to have a transient inhibitory influence, albeit weak, on enzymatic activity in the rod and cone cells [79]. There have been several case reports suggesting a link between sildenafil [80], tadalafil [81] and non-arteritic anterior ischaemic optic neuropathy (NAION), even if this latter clinical entity existed long before PDE5-i were developed. However, the mechanism of damage to the optic nerve is not as well understood. It has

been theorised that sildenafil, which works through the NO-cGMP pathway, may alter the perfusion of the optic nerve head by modifying NO levels [82]. Unfortunately, so far, there is no empirical evidence for or against an association between sildenafil (or tadalafil) and NAION. In addition, hypertension and diabetes are known to predispose to NAION development [83]. Other factors include high cholesterol, arteriosclerosis, stroke, cardiac and intraocular surgery, tobacco use, nocturnal hypotension, blood loss, glaucoma, elevated homocysteine and sleep apnea. The association between NAION and hypertension, high cholesterol and diabetes is stronger in individuals aged < 50 years than in older persons [84]. Many of the risk factors for developing NAION also predict the occurrence of ED, such as hypertension, diabetes, hyperlipidaemia and smoking [85,86]. NAION has been reported rarely in men after taking sildenafil or other PDE5-i for ED. Its incidence in men receiving sildenafil treatment for ED was estimated using pooled safety data from global clinical trials and European observational studies. Clinical trial data in > 13,000 men and on > 35,000 patient-years of observation in epidemiological studies, led to an estimated incidence of 2.8 cases/100,000 patient-years of sildenafil exposure. This is similar to estimates reported in general US population samples (2.52 and 11.8 cases/100,000 men aged  $\geq$  50 years). The data cited herein do not suggest an increased incidence of NAION in men who took sildenafil for ED [87].

#### 4. The role of sildenafil in improving endothelial function

The vascular endothelium continues to attract particular interest in relation to cardiovascular disease and its regulation of vascular homeostasis. Endothelial dysfunction can be defined as an abnormal response leading to a reduction in the bioavailability of NO and impaired vasodilatation, and plays a major role in the development of atherosclerosis and acute coronary syndromes [88,89]. The reduced bioavailable NO may also affect platelet aggregation, vascular wall inflammation and smooth muscle cell proliferation. Endothelial dysfunction is associated with many of the risk factors for both cardiovascular disease and ED (e.g., dyslipidaemia, heart failure, DM and smoking). Some of the drugs shown to have a benefit on morbidity and mortality in cardiovascular conditions, such as the angiotensin-converting enzyme inhibitors in heart failure and hydroxymethyl-glutaryl coenzyme-A reductase inhibitors in ischaemic heart disease, have been shown to improve endothelial function. Furthermore, evidence is becoming available to suggest that measures of endothelial dysfunction might have value as prognostic factors for cardiovascular event rates [90,91].

##### 4.1 Sildenafil and cardioprotection

There are data to suggest that sildenafil might be used in reversing endothelial dysfunction. Sildenafil has been found to have functional effects in platelets in the presence of an

NO drive. Sildenafil (1.0 mM) did not affect human platelet aggregation induced by ADP over the concentration range 0.3 – 30 mM. However, sildenafil 1.0 mM significantly decreased the mean 50% inhibitory concentration for the antiaggregatory activity of sodium nitroprusside. Sildenafil has been demonstrated to improve the vasomotor aspect of endothelial dysfunction in patients with heart failure [92] and patients with Type 2 DM [93]. Whether this might reflect an improvement in some of the other abnormal features of endothelial dysfunction, such as platelet aggregation, increased smooth muscle proliferation and leukocyte adhesion, is unknown. It is reasonable to speculate that chronic sildenafil might have a beneficial effect on exercise tolerance in subjects with heart failure and ED, as demonstrated by acute dosing with sildenafil in patients with heart failure [94].

Multiple lines of evidence have highlighted the importance of the vascular endothelium in regulating important mechanisms involved in the pathophysiology of tissue injury induced by ischaemia and reperfusion (IR) [95]. Endothelial cells appear to be more sensitive to IR than myocytes and a state of reduced endothelial responsiveness to specific stimuli may precede (and contribute to) the appearance of IR-induced tissue necrosis during ischaemia [96,97]. Animal studies have demonstrated that exposure to transient ischaemia (i.e., ischaemic preconditioning) and/or specific pharmacological stimuli can modulate myocardial sensitivity to IR-induced injury [98]. So far, confirmative studies in humans are lacking [99]. In a recent study, it has been demonstrated that sildenafil administration can induce potent endothelial protection via opening of  $K_{ATP}$  channels [100]. Further studies are now necessary to investigate the mechanisms of this effect in greater detail and, most importantly, its potential clinical implications.

One of the positive side effects of PDE5-i is the dilatation of epicardial coronary arteries, the improvement of endothelial dysfunction and the inhibition of platelet activation in patients with CAD. This activity also has an intermediate effect on myocardial ischaemia compared with isosorbide dinitrate and placebo [101].

In a series of experiments, Kukreja *et al.* have shown that sildenafil displayed a preconditioning-like protective effect against IR injury in the heart, and this effect involved upregulation of eNOS and inducible NOS, activation of PKC/ERK, opening of mitochondrial ATP sensitive  $K^+$  channels and attenuation of apoptosis by increasing the Bcl-2/bax ratio [102]. As diverse as these mechanisms are, none of them seem to relate to the fact that the known pharmacological action of sildenafil is inhibition of PDE5. In fact, until a recent paper, none of the aforementioned studies had shown any involvement of PDE5. In a recent study [103], PDE5 expression was identified in isolated mouse cardiomyocytes and treatment of these cells with sildenafil protected them against necrosis and apoptosis. These observations led to the conclusion that, in addition to those aforementioned mechanisms, direct inhibition of PDE5 might be another mechanism through

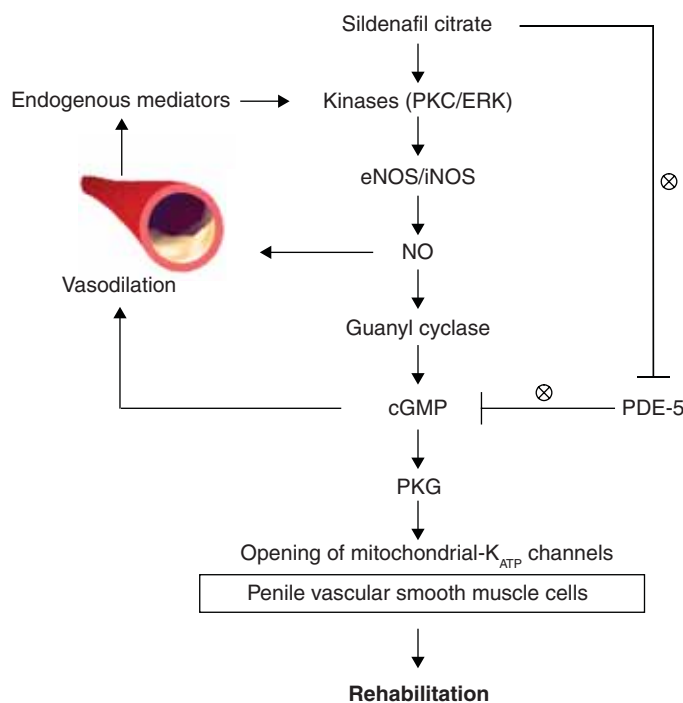
which sildenafil exerts its cardioprotective effect [103]. The distribution of PDE activity has been determined with anti-PDE1 and anti-PDE5 antibodies in the human cardiac ventricle and saphenous vein, and *in vitro* studies were performed on the isolated human cardiac ventricle, CC, saphenous vein and mesenteric artery, as well as on rabbit aorta, dog coronary artery, dog trabecular tissue and rabbit and human platelets. In human CC, the main PDE activity is due to PDE5, sildenafil being a selective inhibitor with a mean  $IC_{50}$  of 0.0039  $\mu$ M. The distribution of PDE5 in the cardiovascular system is consistent with the observed pharmacodynamic and clinical effects of sildenafil. Sildenafil, unlike milirone, a selective PDE3 inhibitor, has no effect on the isolated trabeculae carneae; this is consistent with the lack of PDE5 expression in cardiac myocytes. Sildenafil selectively increases cGMP levels in coronary vascular smooth muscle tissue, but produces no change in cAMP levels, which is consistent with the drug's selectivity for PDE5 [104]. This action appears to be mediated by a sildenafil-induced direct stimulation of NOS via PKC and/or ERK pathways resulting in increased NO generation, guanylate cyclase activation and enhanced formation of cGMP [105]. These results are in keeping with experimental findings suggesting that muscarinic receptors and/or the transduction mechanisms leading to the activation of eNOS are stimulated by a chronic sildenafil treatment [106].

#### 4.2 Sildenafil and the vascular system

Because reduced endothelial function due to inadequate NO release is of critical importance in the development and progression of atherosclerosis and ED [107,108], chronic therapy with sildenafil may represent an important therapeutic strategy for the improvement of endothelial health in diabetics. In fact, NO is the principal peripheral pro-erectile neurotransmitter, and it is released both by parasympathetic nitrenergic autonomic nerves and the sinusoidal endothelium to produce cGMP and relax cavernosal smooth muscle, ultimately resulting in increased intracavernosal pressure [109]. Although prolonged use of sildenafil has been suggested to produce tachyphylaxis [110], other clinical trials including numerous patients followed for longer periods of time have demonstrated only a small (< 5%) patient dropout rate for lack of efficacy [111,112]. These low rates of withdrawal imply that men continued to be satisfied with sildenafil during long-term treatment. Furthermore, tachyphylaxis in most pharmacological and human settings does not occur with drugs that are used in an on-demand basis. Nonetheless, recent data has suggested that sildenafil could have additional and prolonged beneficial effects on endothelial function in diabetic patients if taken on a daily basis [113]. Furthermore, the use of chronic sildenafil could also improve nocturnal erectile activity, thus maintaining the morphodynamic integrity of smooth muscle cells within the CC [114]. A possible concern for tachyphylaxis with sildenafil has arisen with the suggestion that daily dosing may salvage poor responders to on-demand sildenafil therapy. Previous work with human CC

smooth muscle cells in culture reported that tachyphylaxis could occur following repeated exposure to extremely high concentrations of sildenafil (25 mM) [115]. Behr-Russel *et al.*, has hypothesised that animals exposed to chronic treatment could help salvage non-responders to sildenafil therapy. Furthermore, because of its effect on the potentiation of endothelium-dependent cavernosal relaxations, chronic treatment with sildenafil could be of particular benefit in patients with cardiovascular disease-related ED, in which cavernosal endothelial dysfunction occurs [21]. Desouza *et al.*, demonstrated that acute and prolonged low-dose sildenafil treatment has a favourable effect on brachial artery flow-mediated dilatation (FMD) in patients with Type 2 diabetes, acutely as well as after 2 weeks of treatment. Furthermore, after 2 weeks of therapy, this effect is present up to 24 h after the last dose of sildenafil. The improvement in brachial FMD suggests an improvement in endothelial function that represents a near normalisation of this functional parameter in these patients [113]. As already outlined, it might be speculated that the activation of the phosphoinositide-3 kinase/Akt/eNOS phosphorylation cascade could be claimed for this effect, thereby causing a more sustained NO release, vascular relaxation and penile rehabilitation (Figure 1) [116]. Another possible explanation as to why chronic sildenafil may induce endothelial rehabilitation comes from a recent study by Ayala *et al.* that demonstrated improvements in insulin action in a mouse model of diet-induced obesity and insulin resistance [117]. In their experiment, this improvement occurred even in the absence of an exogenous NO donor, suggesting that the endogenous supply of NO in the high-fat-fed state was not limiting to the effect of sildenafil on insulin action. Chronic PDE-5 inhibition also resulted in increased energy expenditure, suggesting that improved energy balance and weight reduction might be partially responsible for the enhanced insulin action without any adverse effects on cardiac morphology or blood pressure measured *in vivo*, supporting human studies that showed no association between long-term use of sildenafil and risk of ischaemic events [118].

The acute effect of sildenafil is not surprising and has been previously demonstrated in patients with congestive heart failure by Katz *et al.* [119]. Nevertheless, it is reassuring to observe this effect in patients with Type 2 DM who are known to have impairment of endothelial dysfunction, which may contribute to both ED and CVD [120]. This data are in agreement with preliminary reports from other authors who demonstrated that daily administration of sildenafil at bedtime for 12 months leads to a significant improvement in penile arterial blood flow and ED regression that persisted after treatment withdrawal [121]. With respect to the known reports of cardiovascular adverse events related to misuse of sildenafil, it is noteworthy to remember that both acute therapy and prolonged therapy were associated with the beneficial effect on the endothelial FMD. Recent data have dispelled initial concerns about an increase in cardiovascular events associated with use of sildenafil [122]. Furthermore, other studies have



**Figure 1. Possible cellular and molecular mechanisms involved in the process of penile rehabilitation.**

eNOS: Endothelial nitric oxide synthase; iNOS: Inducible NOS; PDE: Phosphodiesterase; PKG: Protein kinase G.

demonstrated the lack of detrimental effect of sildenafil in patients with CAD [123], and one study has actually demonstrated haemodynamic improvements in such patients, including increase in coronary flow reserve [124]. The authors personal experience confirms the favourable effects of daily sildenafil (25 mg t.i.d.) in diabetic men with increased cardiovascular risk, regardless the presence of ED. In this preliminary placebo-controlled study, significant improvement in brachial artery FMD was demonstrated, paralleled by a decrease in ET-1 levels, as well as significant changes in surrogate markers of endothelial function [125].

## 5. Conclusion

The clinical significance of PDE5 inhibition is increasingly understood following the pioneering work with sildenafil, and the continuing development programmes for both sildenafil and other marketed inhibitors. Since its initial launch for the treatment of ED, approval has now been granted for treatment of pulmonary hypertension of various aetiologies (REVA-TIO®, Pfizer) [126], and ongoing studies have indicated the potential of PDE5 inhibition for the treatment of a range of additional indications including cardioprotection, memory retention and diabetes. Many of these additional indications are best suited to chronic oral dosing and emphasise the need for highly selective inhibitors with extended duration of action. Experimental findings support the fact that chronic administration of sildenafil may regulate the transduction

pathway leading to the activation of eNOS without loss of effects over the time [21]. More recently, it has been reported that chronic sildenafil exposure determined suppression of cardiac hypertrophy and improvement of heart function in mice exposed to chronic pressure overload [127]. In addition, in a mouse model of diet-induced insulin resistance, high-fat-fed animals treated with sildenafil plus L-arginine or sildenafil alone for 12 weeks had reduced weight and fat mass due to increased energy expenditure [117]. Finally, preliminary data from our study in humans suggest that daily sildenafil added potential benefits on vascular wall remodelling compared to those exerted by concomitant therapies (i.e., angiotensin-converting enzyme inhibitors, sartanes, Ca<sup>2+</sup>-channel blockers or  $\beta$ -blockers), as patients who received placebo showed a progressive decrease of NO-induced vasodilatation over the time despite the use of vascular medications for their comorbidities. Whether these data will extend to humans is not yet known; however, there are encouraging studies reporting that acute sildenafil and vardenafil administration may improve cardiac performance in men with idiopathic pulmonary hypertension [128] as well as endothelial function in diabetic men independently of the presence of ED.

## 6. Expert opinion

Around 9 years after its launch, clinical trial data demonstrated that up to 80% of ED men taking sildenafil (at flexible doses of 25 – 50 – 100 mg) reported improvement in

erections compared with 25% of men taking placebo. Efficacy is present regardless of patient's age, aetiology of ED (organic, psychogenic, mixed) and baseline severity of the condition. Clearly, men affected by severe ED (i.e., with long-standing diabetes, postradical prostatectomy, diffuse atherosclerosis or T-deficiency syndromes) represent very select categories, which may report up to 50% of failures. Recent evidence supports the notion that endothelial dysfunction may be the common causative factor of insulin resistance and Type 2 diabetes [129]. In those patients, a decrease in NO levels along with reducing cGMP production and impaired muscle glucose uptake occurs. The recent demonstration that preventing a decrease in cGMP levels by inhibiting PDE5 intervenes downstream of the site of endothelial dysfunction, which may result in improved insulin action on muscle glucose uptake [117], opens a new scenario in the treatment of diabetic ED. These promising

results are in keeping with those reporting modest reductions in blood pressure, insufficient to stimulate a reflex increase in heart rate and similar for both healthy men and men with CAD or those who use antihypertensive drugs. It is important to remember that sildenafil does not affect the force of cardiac contraction; cardiac performance is unaffected and is mildly vasodilating in the coronary circulation, but does not increase the risk of ventricular arrhythmia. During exercise and recovery, sildenafil does not cause clinically significant alterations in haemodynamic parameters in men with CAD and it has no negative effects on coronary oxygen consumption, ischaemia or exercise capacity [130]. The discovery of improved second-generation PDE5-i (udenafil, avanafil), with enhanced selectivity across the whole PDE family and pharmacokinetics compatible with once-daily dosing will open a new scenario in the treatment of diabetic ED and associated conditions (i.e., obesity and insulin resistance).

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