Endothelial Dysfunction, Erectile Dysfunction and Phosphodiesterase 5 Inhibitors. An Update of the Current Data and Future Perspectives

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Abstract: Endothelial dysfunction is a pathological entity that multiply affects the health status. Erectile dysfunction is being recognized as a condition that is strongly interrelated with endothelial dysfunction, being a vascular event itself. Oral pharmacotherapy for erectile dysfunction has provided us with a new armamentarium on this condition. Phosphodiesterase 5 inhibitors have been investigated and proved useful in clinical practice for erectile dysfunction but in addition to this, the results seem promising of a beneficial effect on endothelial dysfunction, as well.

Keywords: Endothelial dysfunction, erectile dysfunction, phosphodiesterase 5 inhibitors

Introduction

The vascular endothelium consists of a monolayer of specialised, flattened, orthogonal cells lining the inner surface of the blood vessels of any diameter, as well as spaces like the surface of the sinusoids of tissues like the corpus cavernosum of the penis. Its role is regulatory of the vascular tone, coagulation, metabolism and permeability of the vessels.

Endothelial dysfunction results in abnormal regulation of blood pressure, response to inflammation, impairment of the sensitive balance between the vasoconstricting and vasodilating agents and stimuli and coagulation disorders. Endothelial dysfunction is strongly related to hypertension, diabetes melittus (Kirby, 2005), ischaemic heart disease, congestive heart failure (Chong et al. 2003), pulmonary hypertension (Budhiraja et al. 2004) and atheromatosis, but also with diseases like erectile dysfunction and pathological states like lower urinary tract symptoms, benign prostate hyperplasia and bladder outlet obstruction (Rosen, 2006).

Erectile dysfunction is largely a vascular problem, both in the macroscopic and the microscopic level. Excluding hormonal disorders, vascular or neural anatomical defects, it is a process that is directly related to the functional status of the endothelium of the small resistance arteries of the penis and the penile corpus cavernosum. Pathologic conditions like heart disease, high blood pressure, diabetes, atheromatosis, hypercholesterolemia, are strongly interrelated as well as related to erectile dysfunction (Feldman et al. 1994).

From 1998 onwards, a new class of drugs has entered the daily practice, first and mainly in andrology but increasingly also in other specialties like cardiology and pulmonology. These drugs are the phosphodiesterase type 5 inhibitors (PDE5Is). They interfere with the availability of cyclic guanosin monophosphate (cGMP) in the vascular smooth muscle cells, a second messenger of nitric oxide (NO) release from neurons and endothelial cells.

Erectile Dysfunction

The National Consensus Development Panel of the NIH has defined erectile dysfunction as the inability to achieve or maintain an erection sufficient for satisfactory sexual performance (NIH Consensus Development Panel on Impotence, 1993). It has been estimated to affect about 30 million men in the United States, according to epidemiological data from the past decade (Feldman et al. 1994). While in 1995 it was estimated that over 152 million men were having some degree of erectile dysfunction,

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epidemiological projections predict that in 2025 the impotent men will reach 322 million (Ayta et al. 1999).

Physiology of erectile function

Vascular, neurologic, hormonal and psychological factors interact to result in normal erectile function. Erectile function is largely a vasculogenic process, both from the macro- and the micro-anatomical point of view. Tumescence and rigidity of the erect penis is the result of an increased arterial inflow, relative to the venous outflow of blood to and from the sinousoidal structures of the corpora cavernosa. Anatomical and functional integrity of the vascular mechanism of erection (a mechanism that incorporates internal iliac artery, internal pudendal artery, penile artery, bulbourethral artery, cavernous deep penile arteries, helicine arteries within the corpora cavernosa, lateral circumflex arterial branches of the dorsal artery of the penis, corporal sinusoids, deep dorsal, superficial and circumflex veins, cavernous, corporal, emissary veins, periprostatic venous plexous and finaly internal pudendal vein) is of primary importance in the process. Sympathetic and parasympathetic innervation reaches the corpus cavernosum via the cavernous nerves, a branch of the pelvic plexous. Parasympathetic innervation arises from S2–S4 sacral levels, while sympathetic innervation comes from the thoracolumbar (T10–L2) region of the spinal cord. Somatic innervation, bringing proprioceptive and sensory information to the central nervous system is incorporated by the pudendal nerves, which have a different anatomical route from the autonomic innervation (cavernous nerves). A baseline sympathetic tone derived by the interomediolateral gray matter thoracolumbar portion of the spinal cord keeps the penis in the flaccid state when there is no sexual stimulus. Penile arterioles, sinousoidal smooth muscle and endothelial cells receive norepinephrinergic stimulation from the penile adrenergic nerve endings, which result in vasoconstriction and significant resistance in arterial blood inflow. Sexual stimulation induces a parasympathetic (acetylcholine mediated) activity that reverses the vasomotor balance in favor of the vasodilatation. Increased intracellular concentrations of cGMP are responsible for sinousoidal and small arteries smooth muscle relaxation, the molecular mechanism of which is structured over potassium channel-mediated decrease of intracellular calcium concentrations. The increased levels of intracellular cGMP are a consequence of the release of NO both from endothelial cells and nonadrenergic, noncholinergic neurons that end in the presynaptic areas on the vascular smooth muscle. Detumescence is the result of the return of the sympathetic tone on withdrawal of the sexual stimulus and the degradation of cGMP by phosphodiesterase (mainly isoenzyme 5) within the erectile tissue (Figure 1).

Causes and risk factors of erectile dysfunction

Erectile dysfunction may be the result of functional or anatomical impairment of the structures that are involved in the process, in all of the previously, briefly presented levels (from the central nervous system down to the final synapses and from the arteries of the lesser pelvis down to the endothelial and smooth muscle cells of the corpus cavernosum). Arteriogenic erectile dysfunction may result from trauma that disrupts arterial tree integrity at any pre-corporal level. Atherosclerosis is a condition that results similar to trauma, as blood flow in the arterial tree that brings blood into the penis is obstructed. Montorsi et al. in an editorial for European Urology consider erectile dysfunction as the "tip of the iceberg" of a systemic vascular disorder although they pinpoint exceptions to the
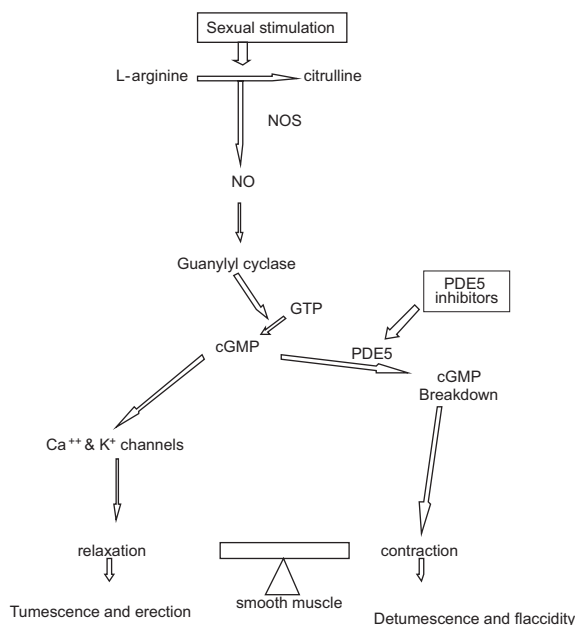
Figure 1. Molecular mechanisms involved in erectile function.

rule like patients with myocardial infarction as the first clinical presentation of coronary artery disease (Montorsi et al. 2003). They consider erectile dysfunction as the clinical manifestation of a disorder involving penile circulation in the same way as angina pectoris is the clinical manifestation of a disorder involving coronary circulation. According to the "artery size hypothesis" that they introduced, at the time that significant vascular obstruction is evident in the smaller size penile arteries, the same plaque burden will not have any interference with blood flow in bigger caliber arteries like coronal arteries, internal carotid or femoral arteries. In an animal model, it has been observed that in a population of hypercholesterol-emic mice with more than 50% occlusion of the iliohypogastric arteries, almost all had erectile dysfunction. But they also noted that in mice with minimal occlusive lesions, 33% had developed erectile dysfunction. That lead to the hypothesis that there may be other factors associated with atherosclerosis and impotence, such as the possible concomitant hypercholesterolemic and atherosclerotic induced alterations in the local reactivity of corpus cavernosum smooth muscle and lacunar space endothelial cells (Azadzoi and Goldstein, 1992). In this way, erectile dysfunction should represent a marker of sub-clinical vascular disease early in the atherosclerotic process. According to Montorsi et al. in a patient with erectile dysfunction, the chance of detecting concomitant coronary artery disease is low, whereas in a patient with clinically evident coronary artery disease, the chance of having erectile dysfunction is high. Also, symptoms of erectile dysfunction should come before symptoms of coronary artery disease (Montorsi et al. 2004). Shared risk factors for erectile dysfunction and coronary artery disease include diabetes mellitus, hyperlipidemia, hypertension and cigarette smoking. The latter may be held responsible for venous leakage (in addition to diseases that affect the macroanatomy of the draining system of the corpora cavernosa, like Peyronie's disease), as it negatively affects the elasticity of the venous wall. In a follow-up study of 9457 patients, a strong association between erectile dysfunction and subsequent cardiovascular disease was found. This association was in the range of risk associated with current smoking or a family history of myocardial infarction (Thompson et al. 2005). In a milestone study on erectile dysfunction and its physiological associations,

supported by numerous references, it is noted that men on treatment for diabetes have a 3-fold probability of erectile dysfunction than non diabetic age-matched controls. Also, a significant correlation was found between erectile dysfunction, heart disease, hypertension and low serum HDL (Feldman et al. 1994). It has also been found that hyperlipidemia, especially high HDL concertrations and total cholesterol/HDL ratio are predictors of erectile dysfunction. Considering that these patients are at increased risk of developing coronary heart disease in the future, they concluded that ED is a sentinel event for coronary heart disease (Roumeguere et al. 2003). Neurogenic causes of erectile dysfunction may constitute neurological disorders affecting the integrity of the nervous pathways (surgery, diabetic neuropathy, spinal trauma). Diabetic neuropathy is also considered to play a role in the pathophysiology of erectile dysfunction, yet another negative final outcome of diabetes mellitus apart of the vascular implications. Diabetics also show a high prevalence of hypogonadism, probably related to their higher Body Mass Index, further leading to erectile dysfunction. They suffer from more severe erectile dysfunction than non diabetic individuals (Corona et al. 2004), and they do not respond well to PDE5I therapy (Vickers and Satyanarayana, 2002).

Vascular Endothelium Physiology and Pathophysiology

The vascular endothelium can be considered an endocrine organ in its own right (Chong et al. 2003) as it plays an active role in functions like hemostasis, fibrinolysis, regulation of vascular tone and permeability and synthesis of growth factors (Lip and Blann, 1997). Factors secreted by the endothelial cells under the influence of a variety of stimuli include NO, endothelins, tissue factor, tissue plasminogen activator and von Willebrand factor. Thrombomodulin, ecto-enzymes, cell adhesion molecules (VCAM, ICAM, selectins), binding sites for factors IX and X, human leukocyte antigen (HLA) are molecules and structures on the cell membrane of the endothelial cells that play several roles in the processes of blood coagulation and anticoagulation, infiltration and oedema and leukocyte adherence. A function largely regulated by the endothelium is vasoconstriction and vasodilatation for the regulation of the vascular tone. Substances which mainly are involved in the process of

Table 1. Main features and roles of the vascular endothelium.

Vasoconstriction	-	Vasodilatation
Hemostasis	-	Fibrinolysis
Regulation of vascular permeability		
Smooth muscle proliferation	-	antiproliferation
Synthesis of growth factors		

vascular smooth muscle tone regulation are NO, endothelin (ET) and, of specific interest to tumescence and erection, cGMP.

Many years ago (Furchgott and Zawadzki, 1980) the role of the endothelium in vasodilatation was demonstrated. The regulation of the vascular tone is largely a function carried out by NO, a gas that some years ago was merely considered to be an atmospheric pollutant. In the endothelial cells, it is produced by two isoforms of the enzyme Nitric Oxide Synthase (NOS), endothelial NOS (eNOS) and inducible NOS (iNOS). NO synthase, acting constitutively or in response to specific signals, catalyzes the formation of nitric oxide from arginine and O_2. Once formed, nitric oxide diffuses only locally through tissues and is highly labile with a half-life of from 2 to 30 seconds. It plays an important role in mediating many local cellular interactions. Release of acetylcholine from adjacent tissues promotes influx of Ca^{2+} into endothelial cells lining blood vessels. After Ca^{2+} binds to calmodulin, the resulting complex stimulates the activity of NO synthase. The nitric oxide that is formed diffuses from the endothelial cell and into neighboring smooth muscle cells where it binds to and activates soluble guanylate cyclase. The subsequent increase in cGMP then leads to muscle relaxation and dilation of the vessel. NO's pivotal role in the maintenance of vascular tone and reactivity is recognized, as it is the main determinant of basal vascular smooth muscle tone, it negates the actions of vasoconstrictors like angiotensin II and endothelin I, inhibits platelet and white cell activation and maintains the vascular smooth muscle in a nonproliferative state (Verma et al. 2003). Physical activation of the endothelial cells by shear stress and pulsatile flow as well as NO release by non adrenergic non-cholinergic neural terminals in the smooth muscle vascular bed (Rand, 1992) is the basis of the vasodilatory action of NO.

On the other hand, a substance also produced by the same endothelial cells that produce NO, endothelin, is a most potent vasoconstrictor. It is 21 aminoacid peptide produced also by other cell types like adrenal cortex cells, smooth muscle cells, renal tubular epithelial cells, glomerular mesangial cells, glial cells, macrophages, mast cells and pituitary cells. Of the 4 isoforms that have been identified (ET 1-4), ET 1 is primarily produced by endothelial cells and acts on the underlying smooth muscle cells (Chong et al. 2003). ET 1 is produced and directly acts on its target receptors, after stimulation by hypoxia, shear stress and ischemia (Cines et al. 1998). Vascular smooth muscle cells, along with other types of cells, express two subtypes of ET 1 receptors, ET_A and ET_B. ET_A mediated actions on the vascular muscle cells include vasoconstriction and smooth muscle proliferation (Newby and Webb, 1996). Increasing vascular smooth muscle cell tone is an action mediated by increase in the intracellular calcium ions (Ca^{++}) concentration. Interestingly, this action persists long after endothelin has dissociated from the receptor, but NO accelerates the restoration of intracellular calcium and shortens the duration of the vasoconstricting effect of ET 1 (Goligorsky et al. 1994). ET 1 and catecholamines potentiate each other's vasoconstricting actions (Cines et al. 1998). ET_B receptors mediate vasodilatation as a consequence of NO and prostacycline release by endothelial cells, but ET_B receptors on the vascular smooth muscle cause vasoconstriction (Verhaar et al. 1998). In endothelial dysfunction, there is an imbalance between the actions of NO and endothelin, due to the decrease in the bioactive concentrations of NO. The vasoconstricting and smooth muscle proliferative actions of endothelin are left unopposed (Lopez et al. 1990).

Cyclic Guanosin Monophosphate (cGMP) is a cyclic mononucleotide that acts as a second messenger for numerous molecular messages in the cell. Synthesis of cGMP is induced by both peptide hormones and NO. Although cGMP was discovered more than thirty years ago, its role in as a second messenger has long been overshadowed by that of cAMP. Synthesis of cGMP is catalyzed by two types of guanylyl cyclase: a soluble cytosolic form and a transmembrane form. Soluble guanylyl cyclases are activated by NO. These enzymes are heterodimers and contain a bound heme molecule that interacts with both subunits. Binding of nitric oxide to the heme leads to a conformational change in the enzyme and stimulates its catalytic activity.

The common mechanism that underlies endothelial dysfunction is oxidative stress. Reactive oxygen species can be derived by enzymatic processes of different types. Final products of superoxide anion (O_2^-) interactions within the endothelium are hydroxyl radicals (HO) and peroxynirite ($ONOO^-$). They either cause cell damage through peroxidation of lipids and sulfydryl groups or regulate several classes of genes, including those controlling the formation of adhesion molecules, chemotactic substances and antioxidant enzymes. They can inhibit the endothelium-dependent vasodilator pathways of NO, prostacyclin and endothelium-derived hyperpolarizing factor (EDHF) and they directly inhibit soluble guanylyl cyclase. They also decrease the activity of calcium-activated potassium channels involved in vasodilatory responses. They have a direct contractile effect on vascular smooth muscle by helping mobilization of calcium and increasing the sensibility of contractile proteins, but they are involved in a number of other mechanisms resulting also in contracted vascular smooth muscle bed (Feletu 2006).

Assesment of endothelial dysfunction

Assessment of endothelial function/dysfunction is an issue of great diversity. The methods that are currently used vary from ultrasound Doppler measurement of the brachial artery diameter and blood flow to quantification of soluble substances produced by the endothelium in plasma or serum. The gold standard is still uncertain, as different approaches to the matter estimate or measure different aspects of endothelial function, dysfunction, activation or damage.

Flow mediated dilatation (FMD) induced by reactive hyperemia has been shown to be endothelium dependent and can be assessed by high-resolution ultrasound in superficial arteries following a well defined and standardized methodology (Corretti et al. 2002; Sorensen et al. 1995) for the non invasive assessment of endothelial function in vivo.

Several molecules have been measured as markers of different aspects of endothelial function. Their evaluation in relation with disease states or outcomes of treatments have been thus far only for research. Their clinical usefulness has not yet been proven or ever more, put in practice. They can offer not only useful diagnostic tests for diseases, but also give a helping hand in assessing the prognosis of pathological states affected by or originating from the vascular endothelium, an organ that is not to be underestimated in its powerfulness to affect almost all functions and anatomical formations of the human body. The major advantage of biochemical measures of endothelial function is that they are inexpensive and offer excellent reproducibility (Verma et al. 2003), as well as a potential role in future mass screening for vascular pathology.

PDE5Is Effects on the Endothelium

Under conditions of sexual stimulation, nonadrenergic-noncholinergic neurons and vascular endothelial cells release NO, which is responsible for increasing levels of cGMP in the smooth muscle cells of the corpora cavernosa of the penis. This is mediated by activation of the enzyme guanylate cyclase. cGMP levels are lowered by a cGMP specific hydrolyzing enzyme, phosphodiesterase (PDE), the isoenzyme 5 of which (PDE5) is found in high concentrations in penile corporal tissue. PDE5 inhibitors maintain high cGMP levels by preventing its degradation, thus promoting tumescence and erection. Three PDE5Is have been marketed since 1998, sildenafil, tadalafil and vardenafil. All three molecules have proven their efficacy in erectile dysfunction treatment (Cirino et al. 2006). They have similarities but also significant differences in pharmacokinetics and PDE isoenzyme selectivity and specificity. These differences are reflected in differences in efficacy and also in safety profiles (Gupta, 2005). Recently, a new mechanism of sildenafil's effect on ED treatment was proposed, that is by inhibition of superoxide formation (by inhibiting NADPH oxidase expression and reducing oxygen free radicals (O_2^-) formation, the amount of bioavailable NO would be enhanced. Adding the inhibitory effect of NO itself on NADPH oxidase, this could constitute of a positive feedback mechanism of reducing the superoxide burden on the endothelium, that is, oxidative stress. They think that their findings could become the basis of repeated dosing of sildenafil, which may reduce intrapenile oxidative stress both in the short and the long term (Jeremy et al. 2005). Acute and also chronic sildenafil treatment has favorable effects on brachial artery flow-mediated dilatation up to 24 h post-dose in men with and without erectile dysfunction. Sildenafil

has been demonstrated to improve the vasomotor aspect of endothelial dysfunction in patients with heart failure (Katz et al. 2000): there was a change in flow mediated dilatation after administration of single doses of 12.5, 25 and 50 mg of sildenafil and the authors concluded that sildenafil improves endothelium dependent vasodilatation in patients with endothelial dysfunction due to chronic heart failure. Another group of investigators evaluated brachial artery diameter as a measure of flow mediated dilatation one hour after a single oral dose of sildenafil 25 mg and also after 2 weeks of daily dosing of 25 mg of sildenafil, 24 hours after the last dose, in type 2 diabetic patients with erectile dysfunction without overt clinical heart disease, and found it significantly improved, in contrast to placebo (Desouza et al. 2002). Other researchers treated men with increased cardiovascular risk with tadalafil 20 mg on alternate days and they found that after 4 weeks they had a significantly improved brachial artery flow mediated dilatation, as well as increased levels of nitrite/nitrate levels and decreased levels of ET 1 at the same time intervals, changes significantly different from their placebo counterparts, even after 2 weeks of tadalafil discontinuation (Rosano et al. 2005), providing optimistic messages of a more sustained effect of chronic PDE5I use on endothelial function in general, not limited to penile tissue. Opinions and findings opposing to those previously described also exist (Robinson et al. 2006). In a study evaluating the effect of sildenafil on altitude-induced hypoxemia and pulmonary hypertension, treatment with sildenafil induced an increase in plasma levels of cGMP (Richalet et al. 2005). Recently, the effects of DA-8159 (udenafil), a novel PDE5I, on endothelial cell, smooth muscle and TGF-beta expression on streptozotocin induced diabetic rats corpous cavernosum was evaluated. Subchronic treatment with DA-8159 prevented the structural degradation of the corpus cavernosum, in terms of reduction of smooth muscle, endothelial cell content, immunoreactivity of TGF-beta 1expression and intracorporeal fibrosis (Ahn et al. 2005). Increased flow mediated dilatation after acute sildenafil therapy was observed in patients with heart failure, a condition characterized by endothelial dysfunction. The same effect was also produced by acute administration of an angiotensin converting enzyme inhibitor, ramipril (Hryniewicz et al. 2005).

Novel Molecules and Therapies of Endothelial Dysfunction

Recently, a method of treating endothelial dysfunction, and oxidative stress was claimed with the administration of D-chiroinositol, D-pinitol and 3,4-di-O-butyryl-D-chiroinositol. The D-chiroinositol and the D-pinitol are claimed to be antioxidants, glucose scavengers, pro-oxidant scavengers, peroxide radical scavengers and superoxide radical scavengers. The D-chiroinositol acts as a glucose uptake promoter and a metabolic normalizer. Administration of 3,4-di-O-butyryl-D-chiroinositol (20 mg/kg) in hyperglycemic and control rats prevented endothelial dysfunction and had a positive effect on microvascular endothelial dysfunction (Larner, 2006).

Conclusions

Erectile dysfunction is a global male population health and quality of life problem. Since 1998, the clinical value of PDE5I's in on demand use has been proved in clinical trials and in everyday clinical practice. ED also is more and more recognized as a problem that is reflective of a more complicate problem: endothelial dysfunction. Therapies that could improve endothelial function may be beneficial to erectile function, as well. Phosphodiesterase inhibition has been shown to positively affect endothelial function. These findings could serve as a first indication of the beneficial results of regular PDE5Is administration to patients with erectile dysfunction, as a means of "endothelial rehabilitation" (Sommer and Schulze, 2005) in our effort to improve or even reverse endothelial dysfunction and restore spontaneous sexual behaviour in patients with erectile dysfunction. Molecular mechanisms of the endothelium, biological markers of endothelial function, genes of interest to the endothelial function are being investigated and the future lies in the effort for a more consistent intervention on endothelial dysfunction.

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