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Review

Molecular Pharmacotherapeutic Targeting of PDE5 for Preservation of Penile Health

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Abstract

The molecular science of erection physiology has established that phosphodiesterase 5 (PDE5) serves an important biological role in the penis. Current research in the field has revealed this molecular effector to be relevant for penile erection, controlling the erectile response by degrading the second messenger product of the erection mediatory nitric oxide (NO) signaling pathway, 3', 5'-cyclic guanosine monophosphate. Accordingly, PDE5 has been targeted for sexual medicine purposes, and orally administered PDE5 inhibitors such as sildenafil, tadalafil, and vardenafil comprise a foremost intervention for erectile dysfunction (ED). New investigation of PDE5 regulation in the penis has suggested alternative roles for the enzyme and new therapeutic opportunities involving its molecular interactions. In particular, PDE5 function is altered under derangements of androgen deficiency, decreased NO bioactivity, and oxidative stress-associated inflammatory changes, thus contributing to an assortment of erectile disorders including hypogonadism-associated ED, recurrent ischemic priapism, penile vasculopathy, and penile fibrosis. This review provides a critical examination of the multifaceted role of the PDE5 regulatory system in the penis and its relevance for applying existing and emerging therapeutic strategies for erectile disorders.

PPhosphodiesterase type 5 (PDE5) is an important molecular player in the biology of penile erection. Acknowledged for its role in controlling the erectile response by degrading the second messenger product of the erection mediatory nitric oxide (NO) signaling pathway, 3',5'-cyclic guanosine monophosphate (cGMP), the enzyme has been targeted for sexual medicine purposes. Presently available orally administered PDE5 inhibitors in the United States (ie, sildenafil, tadalafil, and vardenafil)

comprise a foremost intervention for erectile dysfunction (ED), and they are now considered standard, first-line therapy for this indication (Montague et al, 2005). The advent of PDE5 inhibitor therapy has been momentous in advancing multiple clinical and scientific aspects surrounding this sexual dysfunction. Furthermore, the therapy can be credited with revolutionizing the entire field of sexual medicine, having brought increased awareness and legitimacy to all matters of sexual health across medical and public communities and supporting sexual well being as a foundation for general good health.

Such recent progress not only signifies increasing scientific rigor in the field of sexual medicine, but it also heralds the prospect of multiple new scientific directions that could lead to further therapeutic breakthroughs. This statement aptly applies to a range of disorders of penile erection, beyond the categorization of all erectile impairments generically as ED, classically defined as the inability to attain and maintain an erection satisfactorily for sexual performance (NIH Consensus Conference, 1993). Less well-recognized erectile disorders include hypogonadism-associated ED, recurrent ischemic priapism, penile vasculopathy, and penile fibrosis. Accordingly, the new science of erection physiology implies an expansion in concepts of the pathogeneses of all such disorders and development of specific evidence-based rationales for their effective treatment. The ultimate goal of clinical management for any erectile disorder would be that of preserving erectile function as much as possible and preventing its loss.

In light of PDE5's major involvement in the molecular mechanisms of penile erection, it is timely to conjecture how it may be further exploited beyond its direct pharmacologic inactivation for temporary erectogenesis. One may also surmise that the conventional practice of using PDE5 inhibitors for ED management as a short-term intervention is restrictive, and opportunities likely exist for applying these drugs in various novel ways to derive further penile health benefits. These "outside the box" thoughts are not at all illogical, and in fact, they are consistent with steady advances in the science of penile erection and in the molecular biology of PDE5.

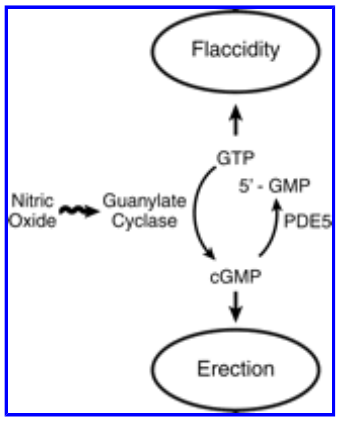

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Figure 1. Schematic diagram of the nitric oxide (NO) signaling pathway in the penis in the context of normal erection physiology. The diagram shows the molecular determinants of a regulatory balance that governs alternative physiologic states of erectile tissue relaxation (erection) and erectile tissue contraction (flaccidity). NO, generated constitutively from L-arginine and released from nerves and endothelium, diffuses to local smooth muscle cells where it primarily activates guanylate cyclase to convert 5'-guanosine triphosphate (GTP) to 3',5'-cyclic guanosine monophosphate (cGMP). By way of cGMP-dependent downstream effector molecule actions, cGMP elicits penile erection. The catalytic function of phosphodiesterase type 5 accounts for degradation of cGMP to its inactive form, 5'-GMP, with subsequent reformation of GTP.

In this review, I examine the multifaceted role of the PDE5 regulatory system in the penis and its relevance for furthering therapeutics for a spectrum of penile health indications. We begin with an overview of the basic biology of PDE5 by highlighting the general properties and molecular interactions of this fascinating molecule. I also briefly describe its familiar characterization in

the penis and summarize the conventional "on-demand" use of PDE5 inhibitors for the clinical management of ED. Then within the context of several specific erectile disorders, I explore the convergence of currently understood PDE5 molecular biologic principles and advancing knowledge of erectile mechanisms. Applying this framework, I discuss the potential pharmacotherapeutic advantages of PDE5 as a molecular target for interventions aimed toward preserving penile health and submit plausible strategies that employ PDE5 inhibitors for this endeavor.

Molecular Biology of PDE5

PDE5 refers to a single phosphodiesterase family belonging to a large superfamily of 11 mammalian phosphodiesterases, also termed metallophosphohydrolases, which function to regulate intracellular cyclic nucleotide signaling (ie, cyclic adenosine monophosphate [cAMP] and/or cGMP) (Beavo, 1995; Francis et al, 2001). Because the enzyme catalyzes the breakdown of cGMP, specifically hydrolyzing the cyclic nucleotide to 5'-GMP, it is termed a cGMP-specific PDE (Rybalkin et al, 2003). Its role is prominently associated with operations of the NO signal transduction cascade (Figure 1). Accordingly, cGMP is the downstream messenger product of NO, generated when the gaseous mediator binds to the iron atom within the heme moiety of guanylate cyclase, which activates it to catalyze the conversion of guanosine-5'-triphosphate to cGMP (Ignarro, 1990). The main function of cGMP is to target protein kinase G, alternatively known as cGMP-dependent protein kinase I (cGKI); this mediator then acts by phosphorylating several proteins in various cells to influence ion channel activity and contractile regulatory protein function (Bender and Beavo, 2006). Because it serves to degrade cGMP, PDE5 understandably exerts considerable regulatory influence over cellular activity. Actions of this enzyme are exerted in key structures in which it is highly expressed, including most smooth muscle tissue and platelets, gastrointestinal epithelial cells, and Purkinje cells of the cerebellum (Francis et al, 2001; Rybalkin et al, 2003; Bender and Beavo, 2006).

The catalytic function of PDE5 explained by its molecular structure. The enzyme features 2 subunits, each having a catalytic domain and a regulatory domain (Corbin and Francis, 1999). The catalytic domain contains a binding site for cGMP, adjacent to which is located the catalytic site of the enzyme. After cGMP occupies the binding site, the phosphate bond of cGMP is catalytically broken, forming linear 5'-GMP. The regulatory domain of PDE5 participates in the regulatory biology of the enzyme. This domain contains allosteric cGMP-binding sites as well as a phosphorylation site (Francis et al, 2002; Okada and Asakawa, 2002). The cyclic nucleotide thus acts not just as the substrate for degradation within the catalytic domain of PDE5 but also binds to the enzyme at its regulatory domain, which serves to elevate the catalytic function of the enzyme. Specifically, this allosteric binding leads to a conformational change of PDE5, exposing a phosphorylation site in the regulatory domain. Following PDE5 phosphorylation by cGKI, PDE5 is further primed to degrade cGMP (Corbin et al, 2000; Rybalkin et al, 2002). This process implies that cGKI heightens the catalytic function of PDE5 by phosphorylating the enzyme as a distinct operation from its effector roles in NO-dependent signal transduction. The conformational change also increases the affinity of the binding sites for the cyclic nucleotide (Gopal et al, 2001). In sum, these regulatory features associated with PDE5 biology indicate a dynamic system of negative feedback controls for the signaling actions of cGMP. The biologic actions of the cyclic nucleotide are therefore tightly controlled because of this system of "checks and balances."

Role of PDE5 in Erectogenesis

PDE5 is prominently expressed in the corpus cavernosum, and thus it is ideally poised to control operations of the NO signal transduction pathway in the penis (Wallis et al, 1999). Because of its direct effect on cGMP availability, it governs actions of the cyclic nucleotide in corporal smooth muscle cells (Gopal et al, 2001). The formation of cGMP involves NO-activated intracellular soluble

guanylate cyclase, following the local release of NO from nerves terminating in penile tissue and both vascular and sinusoidal endothelium of the penis (Ignarro et al, 1990; Burnett, 1995, 2004). Fundamentally, the mechanism of penile erection is a function of corporal smooth muscle relaxation required for blood filling and engorgement of the penis in response to sexual excitement, exerted at the molecular level by cGMP via its effector, cGKI (Andersson, 2001; Mills, 2002; Lin et al, 2005). The opposite function of corporal smooth muscle contraction, which accounts for penile detumescence and flaccidity, involves PDE5. PDE5 then serves to terminate NO/cGMP signaling, along with tonically active contractile regulatory proteins contained in the penis (Andersson, 2001; Mills, 2002; Lin et al, 2005).

Early discoveries of the molecular site of action of PDE5 in the NO signal transduction pathway lent immediate support for the application of PDE5 inhibitors to treat ED (Boolell et al, 1996). By inhibiting PDE5's catalytic degradation of cGMP, these drugs increase intracellular concentrations of the cyclic nucleotide. As such, they understandably exert erectogenic effects under requisite conditions of NO release such as the presence of sexual stimulation. However, their influence is not just a matter of binding to the catalytic domain of PDE5, which results in the blockage of substrate degradation. PDE5 inhibitors also exploit the chemical binding and conformational properties of the enzyme to ensure maximal erectogenesis (Corbin, 2004). By binding to the catalytic domain of PDE5, the drugs serve to block cGMP-elicited negative feedback mechanisms responsible for further cGMP degradation (Turko et al, 1999). Additionally, they stimulate positive feedback for PDE5 inhibition by sequestering cGMP at the PDE5 regulatory domain, an action that promotes the structural binding of PDE5 inhibitors to the catalytic domain of the enzyme (Kotera et al, 2004).

For the clinical management of ED, PDE5 inhibitors have now been established by several consensus bodies to represent appropriate first-line therapy (Padma-Nathan et al, 2004a; Kostis et al, 2005; Montague et al, 2005). In accordance with package labeling instructions, these drugs are recommended to be taken orally approximately 1 hour before anticipated sexual activity with expectations of "on-demand" efficacy in the presence of sexual stimulation. Because their role as erectogenic agents is to potentiate NO-dependent signal transduction of penile erection, optimal responses to therapy require heightened NO release, which occurs with sexual stimulation. As a pharmaceutical class, PDE5 inhibitors have demonstrated comparable sexual intercourse success rates in the average range of 65% to 75%, encompassing various causes and severities of ED (Burnett, 2005d). In addition to demonstrating excellent clinical efficacy profiles, the drugs similarly meet acceptable safety standards (Kostis et al, 2005). Despite these generalities, clinical pharmacokinetic profiles differ among the PDE5 inhibitors in several respects, and these differences may influence patient preferences for one drug over another (Carson, 2006; Wright, 2006). Ongoing studies of the pharmacology of this drug class have centered on such attributes as biochemical specificity and enzyme affinity, which possibly confer superior clinical performances of newer-generation products (Wang et al, 2006; Palmer et al, 2007). For additional clinical information regarding the application of PDE5 inhibitors for ED management, the reader is directed to consult other reviews (Aversa et al, 2006; Carson, 2006; Wright, 2006).

PDE5 inhibitors have been explored for use in health conditions other than ED over the past several years (Schwarz et al, 2007). The demonstration that these drugs produce erectile tissue relaxation has prompted considerations for their therapeutic applications in eliciting relaxation of non-genital tract smooth muscle tissues also expressing PDE5. As the best example, PDE5 inhibitors have gained regulatory agency approval for treating pulmonary hypertension (Barnett and Machado, 2006). Their therapeutic role has also been investigated for other cardiovascular disease states, including Raynaud syndrome, type 2 diabetes, and chronic heart failure (Desouza et al, 2002; Patel and Katz, 2005; Levien, 2006). PDE5 inhibitors have also been shown to exert immune-mediated antitumor

effects, indicating alternative pharmacologic strategies for these drugs (Serafini et al, 2006). Further, PDE5 inhibitors have been associated with endothelial repair (Rosano et al, 2005), and remarkably they stimulate endothelial progenitor cell release from bone marrow (Foresta et al, 2007).

Clinical experience with PDE5 inhibitors has yielded additional insights regarding PDE5 biology. An interesting observation is that adverse effects associated with PDE5 inhibition in various locations of the body also expressing PDE5 lessen over time with the repeated use of PDE5 inhibitors (Carson, 2003). Transiently occurring facial flushing and headaches, for instance, which imply vasodilatory responses of blood vessels supplying the face and head, have been observed to lessen with repeated use of PDE5 inhibitors. These phenomena suggest the occurrence of drug tolerance, defined as the progressive decline in clinical response to a drug following its repetitive and chronic administration (Salva Lacombe et al, 1996). On the other hand, no such evidence of therapeutic resistance has been observed when PDE5 inhibitors are used continuously for pulmonary hypertension (Barnett and Machado, 2006). These different clinical scenarios may be explained by the fact that PDE5 concentrations are biologically high (and presumably less likely altered) in the pulmonary vasculature relative to other structures of the body (Lincoln et al, 1976; Morelli et al, 2004). Varying in content throughout the body, PDE5 also appears to be divergent in its functional roles in various locations, including its interactions with local vascular homeostatic control mechanisms. With respect to the penis, one may infer that PDE5 regulation is potentially modifiable, and pharmacologic manipulation of this system may be directed toward therapeutic purposes.

Clinical Applications

Hypogonadism-Associated ED Treatment— The sex steroidal influence in the physiology of the erectile response has been a topic of controversy for many years. Perspectives have ranged from the position that androgens play a nonessential role in physiologic mechanisms of penile erection (Bettocchi et al, 2004; Handelsman and Zajac, 2004) to the position that these hormones critically influence structural and functional conditions required for the erectile response (Shabsigh, 1997; Lewis and Mills, 2004; Gooren and Saad, 2006; Traish and Guay, 2006).

Interest in androgenic regulatory control of the erectile response led Traish et al (1999) to investigate PDE5 among several biologic mediators of the erectile response. Using a castrated rabbit model, these investigators found that although PDE5 hydrolytic activity in the penis was modestly reduced to a nonsignificant degree by castration, it was significantly elevated after subsequent androgen replacement (Traish et al, 1999). In a subsequent investigation using hypogonadal rabbits, they also showed the importance of the proper androgenic milieu for favorable erectile responses to PDE5 inhibitor treatment (Traish et al, 2003). Morelli et al (2004) contended that male sex steroids accounted for a differentially greater expression of PDE5 in structures of the male as opposed to the female genital/reproductive tracts. These investigators further established that PDE5 gene and protein expression levels, PDE5 hydrolytic activity in both rabbit and human corpus cavernosa, and penile erection functional responsiveness to PDE5 inhibitor treatment in the rabbit were significantly reduced after androgen deprivation but completely restored by testosterone administration (Morelli et al, 2004). Similar findings were demonstrated by this same investigative group applying hormonal manipulation and PDE5 inhibitor administration studies in a rat model (Zhang et al, 2005).

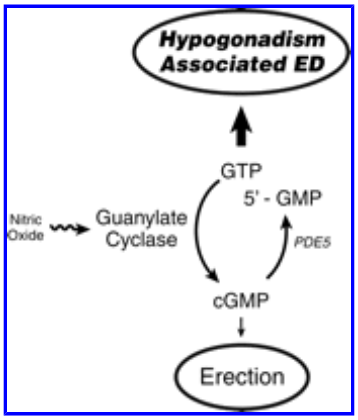

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Figure 2. Schematic diagram of the nitric oxide (NO) signaling pathway in the penis in the context of hypogonadism-associated erectile dysfunction (ED). The diagram depicts a disturbed regulatory balance that predisposes reduced normal erections. The pathophysiology features low constitutive NO bioactivity and dysregulated phosphodiesterase type 5. Androgen deficiency affecting other erectile mechanisms also contributes to ED.

Clinical studies have also indicated the direct influence of androgens on erection responses to PDE5 inhibitors. Guay et al (2001) initially reported that hypogonadal men with ED were poorly responsive to PDE5 inhibitor treatment relative to eugonadal men. Other investigators conducting formal clinical trials in androgen-deficient men confirmed these observations and found that with androgen replacement, these men displayed improved responses to the ED treatment (Aversa et al, 2003; Shabsigh et al, 2004; Yassin et al, 2006). It is noteworthy that these studies in general had shortcomings, such as limited enrollment, which suggests that the results may not be representative of the broadly affected population; relatively short-term intervals of study, which does not affirm the durable effects of this combination therapy; and inclusion of borderline hypogonadal men as study participants, which reduces support for hypogonadism as the primary etiologic attribution for ED in these cases.

Despite these concerns, the preclinical and clinical studies of this subject, taken together, strongly suggest that androgens positively regulate expression and functional activity of PDE5 (including responsiveness to PDE5 inhibitors) in the penis. Further support for this concept can be derived from scientific knowledge of the molecular basis for PDE5 expression. Lin et al (2002) contributed greatly to this knowledge base, describing 2 alternate promoters that regulate transcription of 3 *PDE5A* isoform mRNAs, A1, A2, and A3. They confirmed that the 5'-flanking region of the *PDE5A* promoter contains a consensus sequence for the androgen receptor (Lin et al, 2001). These data provide a molecular explanation for how androgens would modulate PDE5 functional expression and influence the utility of PDE inhibitors to elevate cGMP-induced erectile tissue relaxation (Figure 2).

This model of endocrinogenic ED suggests that androgens sufficiently promote penile erection at the level of the principal molecular pathway mediating penile erection and that they confer benefit when PDE5 inhibitors are used to treat hypogonadism-associated ED. It is also known that androgens exert direct effects on the function of constitutive NO synthetic enzymes (Reilly et al, 1997; Marin et al, 1999). An interesting insight is that although androgens induce PDE5 up-regulation in penile tissue (which would appear to constitute a molecular mechanism aggravating erectile function), they paradoxically foster a "supersensitized" biochemical condition for enhanced erectogenesis by PDE5 inhibitors. The application may move forward with additional clinical trials that aim to define the utility of combined androgen replacement and PDE5 inhibitor therapy. However, a relevant concern is that the definition of the testosterone threshold for indicating symptomatic androgen deficiency in

hypogonadal patients with ED remains to be established. Much of the problem lies in the fact that a wide range in individual thresholds exists for androgen deficiency symptoms (Kelleher et al, 2004). Additionally, the critical threshold of androgenic stimulation required for optimum PDE5 molecular signaling at the penile tissue level remains unknown. While further study of these areas is awaited, an interim clinical management approach may be to institute androgen replacement in combination with a repeat trial of PDE5 inhibitor therapy for any man with ED lacking major risk factors for such hormonal treatment and who had failed earlier PDE5 inhibitor treatment. The approach should be applied while appropriately monitoring testosterone levels and assessing therapeutic benefit.

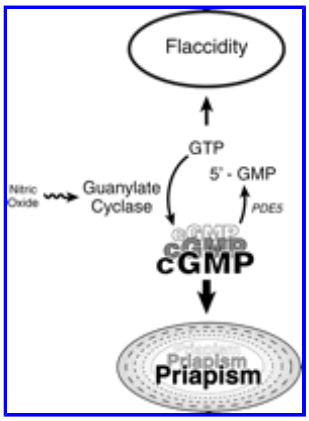

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Figure 3. Schematic diagram of the nitric oxide (NO) signaling pathway in the penis in the context of recurrent ischemic priapism. The diagram depicts a disturbed regulatory balance that predisposes excessive penile erection. The pathophysiology features low constitutive NO bioactivity basally and dysregulated phosphodiesterase type 5. Upon periodic heightened NO generation and release, 3',5'-cyclic guanosine monophosphate is not degraded and accumulates, causing an excessive erectile tissue vasorelaxant response.

Management of Recurrent Priapism— The mention of priapism, an erectile disorder of prolonged, nonwillful, and often painful penile erection, to many clinicians evokes thoughts of a vexatious clinical situation. These clinicians may have encountered distressed patients presenting typically in emergency room settings, often repeatedly, who were required to undergo such torment as open surgical drainage procedures of the penis, even though these measures often fail to avert the permanent structural and functional damage associated with this ischemic "compartment syndrome" (Burnett, 2005c). These phenomena are common in patients with sickle cell disease and other hematologic dyscrasias, differing from priapism associated with pharmacologic complications, lower genitourinary tract solid or hematogenous malignancies, and genital trauma. Because of a poor understanding of recurrent priapism from a pathophysiologic standpoint, effective management consisting of mechanism-specific treatments offered when needed or preferably in a preventative manner has remained lacking.

Insight into the pathogenesis of the problem initially followed the observation that mice genetically engineered with deletion of the endothelial NO synthase (eNOS) gene, hence termed eNOS knockout mice, exhibited excessive erection tendencies (Burnett et al, 2002). Subsequent molecular investigation of these mice and mice transfected with the human gene for sickle cell disease also displaying a priapism phenotype revealed that because of the altered basal NO signaling in their penises, both transgenic mouse models had developed a down-regulation of the penile expression and activity of PDE5 (Champion et al, 2005). These data suggested that uncontrolled penile erections result from the relatively diminished function of PDE5. In specific terms, following the heightened release of NO neuronally during prolonged sexual stimulation or in association with sleep-related erectile activity, known circumstances preceding priapism occurrences, cGMP is produced and its

amount surges; excessive erectile tissue relaxation is caused precisely because of basally insufficient functional PDE5 to degrade the cyclic nucleotide (Figure 3).

A homeostatic regulatory role of NO in the penis is further evinced by the fact that the expression and activity of other molecules downstream from and interacting with the NO signaling pathway also are downwardly adjusted under conditions of reduced tonic NO signaling. For instance, the main subcellular system responsible for erectile tissue contraction, the RhoA/Rho kinase signaling pathway, is underactive in this context and provides a permissive basis for priapism to occur in the presence of a vigorous erectile stimulus (Bivalacqua et al, 2007). Additional oxidative/nitrosative mechanisms, which decrease NO signaling function in the penis, are believed to contribute to the pathophysiology of priapism (Munarriz et al, 2003). PDE5 down-regulation has also been shown in cultured cavernous smooth muscle cells of rats (Lin et al, 2003) and humans (Vignozzi et al, 2006) under hypoxic conditions that mimic the penile ischemia of priapism.

These scientific discoveries have served a clinical translational purpose. Because PDE5 dysregulation underlies recurrent priapism, a reasonable assumption is that the reversal or prevention of this molecular disturbance in the penis for those individuals afflicted by the erectile disorder would be therapeutically advantageous. As additional research work has suggested, PDE5 function in the penis is upwardly changeable after an ample duration of PDE5 inhibitor treatment. Musicki et al (2005a) established elevated PDE5 expression and activity in the penis after chronic dosing of the PDE5 inhibitor sildenafil in an in vivo rat experimental paradigm. Lin et al (2003) also confirmed up-regulated PDE5 protein levels in rat cavernous smooth muscle cells as well as *PDE5A* promoter activity in the monkey fibroblast cell line COS-7 transfected with plasmid constructs carrying *PDE5A* promoters, following sildenafil administration in both experimental protocols. These data provided scientific support to use PDE5 inhibitors as a treatment for recurrent priapism. In preliminary studies of this hypothesis, we found that a precise regimen of continuous, long-term PDE5 inhibitor treatment that is unassociated with sexual stimulation alleviated further episodes of the disorder in several men with recurrent priapism (Burnett et al, 2006). Further, we found that this treatment did not alter normal erectile function required for satisfactory sexual activity (Burnett et al, 2006).

Scientific knowledge of the mechanisms involved in PDE5 regulation importantly lends biologic coherence for this therapeutic application. This support derives from the role of feedback control mechanisms exerted by downstream components of the NO signaling pathway, which influence the biologic function of PDE5. In their studies of *PDE5* gene regulation, Lin et al (2002) identified cyclic nucleotide-inducible promoters as well as adjacent enhancers preceding the 3 *PDE5A* isoform mRNAs present in human penile cavernosum. These promoters confer additive responsiveness to the basal promoter, demonstrated by increased *PDE5A* promoter activity in promoter activity assays following the administration of cGMP or cAMP (Lin et al, 2002). Consequently, the inducibility of *PDE5A* promoters by cyclic nucleotides suggested that increased cGMP levels in the penis resulting from the use of PDE5 inhibitors increases the expression and activity of PDE5 according to principles of reciprocal regulation of the enzyme.

The current molecular science of recurrent priapism yields a number of important insights. The erectile disorder underscores the pertinence of PDE5 in the penis as a regulatory component of erection physiology, ensuring that erections do not persist uncontrollably. In this respect, PDE5 can be viewed as having a major impact on penile health by preserving physiologic erectile activity and limiting pathologic consequences of ischemia and reperfusion injury associated with prolonged erections. Also featured in this disorder is the condition of a low but modifiable PDE5 content, similar to other smooth muscle-containing structures in the body expressing limited amounts of PDE5.

This basal condition in the penis in association with priapism is pathophysiologic, and PDE5 up-regulation with pharmacologic treatment is aimed toward achieving physiologically normal regulatory conditions in this organ. According to current investigative work, recurrent priapism occurs because basally low NO bioavailability predisposes reduced PDE5 function in the penis (Champion et al, 2005). This corollary suggests that investigators continuing work in the field should bear in mind the potential therapeutic goal of addressing the primary pathophysiologic element of the disorder.

Concerning the use of PDE5 inhibitors for this disorder, it is recognized that the therapy is not ideal. Requirements of this therapy include strict adherence to a pharmacotherapeutic regimen, which must be followed to avert provoking a priapism episode, and endurance of a delayed clinical effect, which may be as much as several days or more before PDE5 is favorably regulated, based on early clinical experience (Burnett et al, 2006). In light of these situations, an argument could be made that PDE5 agonist therapy offers advantages as an immediately active intervention in the event of a presenting priapism episode. Perhaps further efforts can be given to researching and developing this specific treatment. However, corrective treatments inarguably remain the highest form of intervention, and future therapeutic strategies for this disorder may well be directed toward effecting durably normative NO signaling and PDE5 regulation in the penis.

Penile Vascular Protection— The medical literature widely supports vascular health objectives as a means toward achieving long-term health maintenance and longevity. Several thought leaders in sexual medicine have further pointed to penile vascular health as a critical gauge of this outcome (Kloner et al, 2003; Solomon et al, 2003). The pathogenesis of vascular disease both systemically and locally in the penis is linked with NO imbalance via endothelial defects and/or oxidative stress, which subsequently diminishes the physiologic actions of NO and its effectors (Cooke and Dzau, 1997; Bonetti et al, 2003) (Figure 4). To address this pathophysiology, suggested preventative practices have been advocated to include increasing physical fitness, improving healthful dietary habits, and reducing obesity (Esposito et al, 2004; Esposito et al, 2006). Additional consideration has been given to medical therapies such as regularly used PDE5 inhibitors under the premise that this treatment may afford long-term vascular healthful benefits for the penis (Montorsi et al, 2000; Burnett, 2005a).

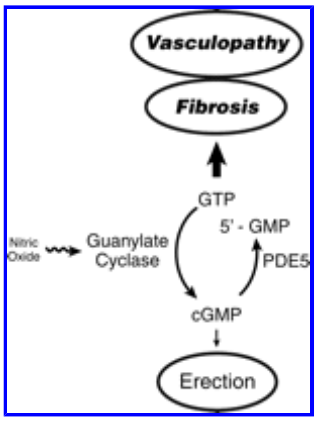

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Figure 4. Schematic diagram of the nitric oxide (NO) signaling pathway in the penis in the context of penile vasculopathy and penile fibrosis. The diagram depicts a disturbed regulatory balance that predisposes reduced normal erections. The pathophysiology features low constitutive NO bioactivity. Pathologic conditions associated with these erectile disorders contribute to erectile dysfunction.

Basic scientific evidence supports a penile vasculoprotective premise associated with long-term PDE5

inhibitor use. Several scientific studies have shown the utility of chronically applied PDE5 inhibitors in improving the structure and function of the cavernosal tissue and provided plausible mechanisms for these beneficial effects. In experimental paradigms involving rats that were chemically diabetogenic (De Young et al, 2003; Ahn et al, 2005), intact (Behr-Roussel et al, 2005), or aged (Musicki et al, 2005b; Ferrini et al, 2007) or had cavernous nerve injuries (Vignozzi et al, 2006; Ferrini et al, 2006a; Lagoda et al, 2007), continuous systemic PDE5 inhibitor treatment ranging from several days to 3 months preserved erectile tissue morphology and erection physiology to a better extent than did control treatments. Improved erectile responses were found to be sustained after confirmed drug clearance or withdrawal of active drug for in vivo (Musicki et al, 2005a; Lagoda et al, 2007) and in vitro (Behr-Roussel et al, 2005) protocols, respectively. Foremost possible biologic mechanisms by which PDE5 inhibitors afford penile vascular protection include antioxidation (De Young et al, 2003; Lagoda et al, 2007), antiapoptosis (Ahn et al, 2005; Musicki et al, 2005b), and activation of blood flow-associated vasodilatory effectors (Behr-Roussel et al, 2005; Musicki et al, 2005b; Ferrini et al, 2006a; Vignozzi et al, 2006; Ferrini et al, 2007).

A growing body of clinical literature also suggests that chronically used PDE5 inhibitors exert sustained healthful effects on the penile vasculature. Efficacy and tolerability have been demonstrated for sildenafil and tadalafil using once-daily or alternate day dosing regimens in men with ED enrolled in uncontrolled, open-label design studies (McMahon, 2004; Caretta et al, 2005; McMahon, 2005; Mirone et al, 2005; Sommer and Schulze, 2005; Buvat et al, 2006). In clinical trials with the rigor of randomization and placebo control design, efficacy and safety end points have also been shown for tadalafil (Porst et al, 2006; Rajfer et al, 2007). A substantial groundswell of interest has been generated to apply this mode of therapy to the postradical prostatectomy population according to a conceptual "penile rehabilitation" strategy. This population experiences at least some temporary degree of ED as a consequence of the surgery, even when cavernous nerve-sparing techniques are applied (Burnett, 2005b). Much attention was paid to the placebo-controlled study involving postoperative nightly administration of sildenafil, which found a 27% return of spontaneous, normal erectile activity rate compared with the 4% rate found in the placebo arm at 1 year after surgery (Padma-Nathan et al, 2004b). In other reports involving open-label study designs, investigators attempted to define an optimal therapeutic regimen in terms of such variables as dosing schedule, duration of administration, and timing of application while also describing improvements in spontaneous erectile function resulting from chronic PDE5 inhibitor use (Gontero et al, 2005; Mulhall et al, 2005).

Several caveats should be addressed in considering the utility of long-term PDE5 inhibitor treatment for this clinical application. One early identified controversy was whether the therapeutic strategy could cause pharmacologically induced "tachyphylaxis," as suggested by a report that described a 20% dose elevation rate and 17% discontinuation rate due to loss of efficacy in patients with ED using sildenafil "on demand" over a 2-year interval (El-Galley et al, 2001). However, likely explanations for declines in treatment effect over the long term are underlying disease state progression, inadequate dosing and application of the therapy, relationship difficulties, and psychogenic factors (Steers, 2002). Furthermore, findings of consistent efficacy and tolerability of treatment following both long-term, "on-demand" schedules reported previously (Carson, 2003) and long-term, continuous dosing reported more recently (Porst et al, 2006; Rajfer et al, 2007) argue against the development of treatment tolerance. It is acknowledged that PDE5 expression levels apparently increase with continuous treatment in basic science experimental paradigms (Lin et al, 2003; Musicki et al, 2005a), but the consequence of increasing PDE5 expression above normative levels in the penis was not demonstrated to negatively impact physiologic erectile responses (Musicki et al, 2005a; Behr-Roussel et al, 2005).

Another matter for consideration is whether prophylactic PDE5 inhibitor therapy should be given only to patients with certain underlying medical conditions. Much interest exists to apply the therapy to patients with severe forms of ED or conditions that would predict the likely development of ED. In this vein, the therapy would apply to those individuals with such risk factors as diabetes, cardiovascular disease, prior pelvic surgery, and aging. In their rat model study of chronic sildenafil dosing, Musicki et al (2005b) found that erections improved only in erection-impaired, aged rats but not in erection-intact, young rats. Compensatory homeostatic mechanisms were found to develop in young rats, suggesting to the investigators that such mechanisms are operable in the penis of the "healthy" individual in response to long-term PDE5 inhibitor treatment which limit supernormal erectogenic effects and concurrently prevent potentially harmful excessive erections.

Continued study is needed to confirm clinical impressions of a penile vascular protection benefit, which at this stage should be considered preliminary. Additionally, further investigation is needed to clarify molecular mechanisms associated with the presumed therapeutic benefit. Investigative work done in the cardiovascular field has shown the preconditioning effects of PDE5 inhibition against ischemic/reperfusion injury in the intact heart (Das et al, 2002; Kukreja, 2007). Cardioprotective effects have been related to activation of protein kinase C/extracellular signal-regulated kinase signaling, opening of mitochondrial adenosine triphosphate-sensitive potassium channels, and attenuation of cell death resulting from necrosis and apoptosis (Kukreja, 2007). Further investigation may reveal whether or not such cellular or subcellular effects actually occur in the penis following long-term PDE5 inhibitor treatment. It is recognized that advancing the knowledge base in this area may occur most readily at the experimental animal model level, in which penile tissues are more easily obtained for molecular studies and objective erection testing is also more feasible. However, important inferences may still result from continued active research efforts expended at the clinical level. Recent work in men by Foresta et al (2007) showing that vardenafil increases circulating progenitor cells, which are involved in the process of neovascularization and continuous repair of the endothelium, via bone marrow stimulation has contributed to defining the endothelial protective role of PDE5 inhibitors.

Penile Tissue Health Restoration— It is well documented that penile fibrotic conditions arise in association with penile trauma, prior radical prostatectomy, priapism, and idiopathic circumstances, which generally refer to Peyronie disease. All of these conditions represent pathologic changes of the penile tissue that interfere with the functional activity of the NO signaling pathway (Figure 4). Recent interest has been generated to modulate the pathway as an approach for correcting these conditions.

Support for the hypothesis has come mainly from basic science investigation. Valente et al (2003) described the counteraction of the penile fibrotic process in a rat model of Peyronie disease using the NO precursor L-arginine and both the PDE4 inhibitor pentoxifylline and the PDE5 inhibitor sildenafil. This same research group also observed that penile fibrosis is prevented in rat models of Peyronie disease (Ferrini et al, 2006b) and cavernous nerve injury (Ferrini et al, 2006a) following administration of vardenafil and in an aging rat model following administration of sildenafil (Ferrini et al, 2007). These studies do suggest the commonality of the pathogenic changes with respect to different diseases encountered clinically. The investigators have repeatedly confirmed that PDE5 inhibitor treatment for these penile injuries increases the number of cavernosal endothelial and smooth muscle cells, in essence restoring the normal structure of the penis. They suggested that at a molecular level, the ameliorative effect of modulating the NO signaling pathway results from the actions of its components cGMP or cGKI against the mitogen-activated protein kinase system or other cytokine-dependent oxidative stress mechanisms involved in penile fibrosis (Valente et al, 2003). In further support of the NO influence in correcting penile fibrosis, this group also

determined that PDE5 inhibitor treatment increases expression of the inducible form of the NO synthase enzyme (iNOS), which supposedly releases NO at levels contributing to healthful effects in the penis (Ferrini et al, 2006a; Ferrini et al, 2006b; Ferrini et al, 2007). However, the exact mechanism for iNOS induction resulting from elevated cGMP levels awaits further clarification. Other investigators have also shown that PDE5 inhibitor therapy counteracts oxidative stress in the penis. Koupparis et al (2005) showed that sildenafil reduced both superoxide formation and the expression of $gp47^{phox}$, a subunit of the reactive oxygen species source NAD(P)H oxidase in rabbit cavernosal smooth muscle cells exposed to an analog of the cytokine/vasoconstrictor thromboxane A_2.

Clinical support for the hypothesis is currently limited. Rajfer et al (2006) recently described 2 patients with penile fibrosis resulting from major priapism episodes whose priapism resolved after taking the PDE inhibitors pentoxifylline and sildenafil according to a daily treatment regimen over 1 year. The preclinical and clinical results thus far are encouraging and should stimulate further interest in studying the potential use of PDE5 inhibitors as penile antifibrotic agents.

Summary

The significance of PDE5 in the penis is well understood in terms of its role in penile erection. There is ample evidence that this enzyme serves an important regulatory role for this biologic function. However, increasing attention has been given recently to the regulatory basis of PDE5, which influences its operation in the penis. This concept implies that the regulatory determinants of PDE5 biology in this organ are as important for the mechanistic effects of PDE5 as its biologic activity alone. Regulators in this regard include both endogenous and exogenous factors. Endogenously, androgens and upstream components of the NO signaling cascade affect PDE5 expression and activity in the penis. Derangements in their actions account for pathologic consequences in the penis, and conversely interventions such as exogenous androgen replacement or pharmacologic optimization of NO signaling in the penis using PDE5 inhibitors improve or restore penile physiology. The current understanding that PDE5 biology in the penis is not static but rather is modifiable and subject to various forms of modulation suggests that the enzyme is an opportune pharmacotherapeutic target for preserving penile health. Ongoing investigation in the field may suggest additional innovative strategies that may be specifically applied to advance this health objective.

Footnotes

Read in part at the postgraduate course, "Issues in Male Sexual Health: From Benchwork to Bedside," American Society of Andrology 31st Annual Meeting, Chicago, Ill, April 8, 2006, with appreciation to Dr Wayne J.G. Hellstrom, Course Director.

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