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PERSPECTIVE

The two phases of the clinical validation of preclinical translational mechanistic research on PDE5 inhibitors since Viagra's advent. A personal perspective

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Abstract

The FDA approval of Viagra (sildenafil) for the on demand treatment of erectile dysfunction (ED) through relaxation of the corporal and cavernosal vascular smooth muscle that results in an increase in blood flow to the corporal tissues stemmed from 2 decades of research, mainly at academic centers. This culminated in the finding of the nitric oxide/cGMP pathway as the mediator of penile erection, followed by some years of basic studies and clinical validation at Pfizer. Further on, new translational laboratory and animal research from our group initiated a second phase when we proposed an alternative therapeutic schedule and mechanism of action for PDE5 inhibitors (PDE5i) in both corporal veno-occlusive dysfunction (CVOD) and Peyronie's disease (PD), specifically, continuous long-term administration (CLTA) to achieve sustained levels of cGMP within the penis. Due to the extended half-life of the long-acting PDE5i, tadalafil, this new alternative encompasses preferentially daily administration, although shorter half-life PDE5i, like sildenafil and vardenafil work too, depending on the duration, dose, and frequency of their administration This novel use was initially supported by showing the antifibrotic/antioxidant effects of nitric oxide and cGMP, produced by the induction of iNOS, as a mechanism of defense against collagen deposition in the localized fibrotic plaque of PD in an avascular tissue, the tunica albuginea. Our studies on iNOS and the progressive diffuse fibrosis occurring in the smooth muscle in CVOD, led to proposing the CLTA of PDE5i for maintaining sustained cGMP levels both in PD and in CVOD in order to halt or regress the penile fibrosis. In CVOD, we showed that PDE5i protect the corporal smooth muscle and reduce myofibroblast activation and number, counteracting the underlying corporal tissue pathology that causes CVOD, and potentially ameliorating long-term CVOD or even curing it. This review is focused on this novel PDE5i anti-fibrotic therapeutic concept.

The first phase: the vasodilator action of on demand administration of PDE5 inhibitors for erectile dysfunction (ED)

Twenty years ago, when Viagra, the brand name of sildenafil, received FDA approval, a true revolution started for the treatment of ED and in sexual medicine. This approval was, however, the culmination of prior extensive translational research that led to the identification of nitric oxide (NO) as (a) the endothelial-derived relaxing factor (EDRF) and its role on the activation of guanylyl cyclase to produce the vasodilator cGMP, and (b) the mediator of non-adrenergic non-cholinergic neurotransmission and penile erection [1]. This discovery resulted from the application of pharmacology to systemic vascular and neurological systems, blended with cellular and molecular biology studies, and finally with a focused urological insight. This was a fruitful continuation of at least a prior decade of experimental studies in animal models and patients that had led to the definition of the main physiological and pharmacological basis of penile erection. From these studies came the discovery of the mediator of the erectile response, and subsequent findings that clarified this mechanism [2]. These discoveries opened up the way for the application of Viagra and phosphodiesterase 5

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inhibitors (PDE5i) as a way to treat erectile dysfunction (ED).

These research endeavors were developed initially in academic centers. When combined with what was known about the PDE5 enzyme responsible for the breakdown of cGMP in the vascular system, it did not take long for the pharmaceutical industry to begin looking for products that would fulfill this niche. It was Pfizer's luck that they reportedly had a product that clinically seemed to fulfill the role of this mechanism of action, i.e., enhancement of erectile function. This phase led to both the first clinical trials sponsored by Pfizer and the immediate impact of Viagra on sexual medicine practice, and it may well have had some bearing on Dr. Louis Ignarro sharing the Nobel Prize of Physiology or Medicine for 1998.

These findings were an outstanding example on how basic laboratory and animal research together with the clinical observations in man spurred a major advance in medicine, while at the same time establishing a clear mechanism of action for sildenafil based on smooth muscle relaxation, which acted as the prototype for the development of other PDE5i. This mechanism of action is what is termed the "on demand" treatment of ED by the patient. The PDE5 inhibitor facilitates an erection by enhancing the smooth muscle relaxation of both the corporal tissue and the cavernosal arteries that have initially undergone some relaxation as a result of sexual stimulation. It is the decrease in peripheral resistance due to corporal smooth muscle relaxation as well as relaxation of the smooth muscle within the arterial media that increases blood flow to the corporal sinusoids, thereby filling them with blood.

The accepted mechanism of action for Viagra or any other PDE5i is to transiently maintain adequate levels of cGMP by inhibiting PDE5. Sexual stimulation itself elicits a response by the brain cortex and hypothalamic centers resulting in NO production by neuronal nitric oxide synthase (nNOS), and this nitrergic signal is transmitted through the spinal cord to the cavernosal arterial and corporal smooth muscle where guanylyl cyclase is activated by NO released from the nerve terminals, yielding cGMP as the ultimate smooth muscle relaxant [1, 2]. Therefore, this traditional "on demand" PDE5i practice was at the time of Viagra's approval for ED, and still is, a short-term approach that ameliorates a symptom—the inability to initiate and maintain a transient erection for sexual activity—but does not treat the underlying cause(s). In fact in the late 1990s the corporal smooth muscle pathophysiology was not well known, often assumed for NO to be of mainly endothelial origin, and not the tissue target of the on demand treatment with Viagra, and by extension, PDE5i. In summary, the PDE5i were and are administered to facilitate a transient palliative tissue relaxation, and not as a curative treatment.

Upon the full entrance of Viagra in clinical practice from 1998 onwards, the research on PDE5i was dominated by pharmaceutical companies focusing on clinical trials, and some on the test of alternative PDE5i, which in turn modified the outcomes that urologists had so far used for measuring the effectiveness of pharmacological treatment of ED. The International Index of Erectile Function (IIEF) and other questionnaires became the prevalent, or often the virtual single tool, over standard medical procedures such as the histopathology of penile corpora biopsies, Doppler ultrasound for blood flow, rigiscan for hardness of erection, and erectile response to injected vasoactive drugs into the corpora, that had been extensively used for 2 decades for assessing more precisely and mechanistically ED. Simultaneously, funding for basic science laboratory and animal studies on PDE5i and ED from NIH and other agencies decreased, while pharmaceutical and other private companies focused instead on human studies.

The second phase: the antifibrotic/smooth muscle protective action of continuous long-term administration (CLTA) of PDE5 inhibitors for corporal veno-occlusive dysfunction (CVOD) and Peyronie's disease (PD)

Despite this situation, an alternative type of research on PDE5i emerged in academic institutions after the introduction of Viagra. These studies focused on laboratory and animal research and seemed more innovative than what was occurring with the human studies. One of these lines of translational research led in the early 2000s to the proposal of a distinct therapeutic application of PDE5i, the treatment of corporal veno-occlusive dysfunction (CVOD) and Peyronie's disease (PD). This was based on the effects of an oral continuous long-term administration (CLTA) of PDE5i to maintain a sustained level of cGMP in the (a) penile corporal tissue, with the goal of halting or reversing smooth muscle fibrosis in the corpora cavernosa, then starting to be accepted as the cause of CVOD, and (b) tunica albuginea, with the goal of inhibiting the fibrosis in the PD plaque. Corporal fibrosis in particular is known to affect over 2/3 of patients with ED, by impairing the relaxation of corporal smooth muscle and consequent compression of the veins against the rigid tunica albuginea that retains the blood in the penis during a rigid erection. A therapeutic treatment of CVOD, which halts or even reverses the progression of the underlying fibrosis, thus also offers a path to permanent or long-term resolution of many cases of ED.

This new CLTA concept for PDE5i was based on a corporal antifibrotic/smooth muscle protective mechanism different from the well-known vasodilation action in the

standard "on demand" administration (virtually exclusive prior to 2008), which just facilitates the development of an erection. In contrast, when used in an anti-fibrotic manner, the aim is to cure the corporal histopathology causing the CVOD, rather than to just alleviate briefly the ED symptom. The proposal in those translational laboratory/animal studies [3–20] was therefore a different use of the PDE5i, and was reported prior to the FDA approval in 2008 of a new form of human administration that in turn rapidly impacted ED practice. The latter was the daily use of the long-acting PDE5i, Cialis (tadalafil), based essentially on three clearly stated reasons: (a) being more spontaneous for the patient; (b) having better efficacy/side effects ratio; and (c) aiming to improve blood flow to the penis as on the on demand administration. The daily use of all PDE5i in the clinic was mechanistically and in terms of the objectives just "more of the same" in comparison to "on demand" use.

How did the novel CLTA/antifibrotic/curatively aimed mechanism of PDE5i for CVOD and PD evolve within and from translational basic science? In the early 2000s, it was known that tissue fibrosis, the exaggerated deposition of collagen fibers, is caused by the activation of fibroblasts and a more intensive collagen factory, the myofibroblasts, by factors that were then not well identified. A sort of dysregulated wound healing where various cell types persist elaborating collagen and extracellular matrix (ECM) and coexisting in some cases with the damage or loss of other cell types essential for organ function.

The LABioMed/UCLA group postulated that the localized and relatively fast fibrosis of the tunica albuginea in PD induced by the profibrotic agent TGFβ1 injected into a tunical site in its rat model, was an excellent experimental approach to go in-depth into the etiology and pathophysiology of this process, by defining the effects of agents such as cytokines, chemokines, and reactive oxygen species (ROS) produced by oxidative stress, that elicit the fibroblast/myofibroblast conversion [3]. This paradigm was used for the study of the CLTA antifibrotic effects of PDE5i in the localized PD-like plaque [4–11], and in the corporal fibrosis and subsequent CVOD occurring in rat models for certain risk factors of ED [12–20].

The initial resulting studies in the TGFβ-1 and fibrin-induced models of PD-like plaques and cell culture proved that, contrary to the interpretation of prior reports in the animal model of PD with associated ED [21], inducible NOS (iNOS) was expressed in the penis as a mechanism of *defense* against fibrosis, and not in a pro-fibrotic process,. This was shown to occur by the production of sustained levels of NO that counteract ROS, and cause both inhibition of myofibroblast production and also their apoptosis, and that this occurred in part by cGMP formation. It was proved that CLTA with sildenafil, as well as gene therapy with iNOS cDNA, regressed the PD-like plaque, and this was extended later to prevent or regress the plaque by vardenafil [3–8]. This was very significant because it occurred in an avascular and non-contractile tissue, the penile tunica albuginea, indicating that the increase of blood flow by vasodilation or relaxation did not play any significant role in the anti-fibrosis/CVOD mechanism of the CLTA of PDE5i for PD. We further discussed the implications of this novel therapy for treating PD in several reviews [8–11].

We confirmed that our findings in the PD model were also equally applicable to corporal fibrosis, showing the antifibrotic effects of CLTA of vardenafil, sildenafil, and tadalafil on the diffuse corporal fibrosis, and specifically CVOD, caused by bilateral cavernosal nerve resection in a rat model simulating post-radical prostatectomy, and of sildenafil in regressing both processes in the aging rat model of ED [12–17]. We proposed CLTA of PDE5i as a therapy for PD [10, 11] and CVOD in men [18–20].

In summary, extensive evidence supports CLTA of PDE5i to halt or reverse fibrosis of the penile corpora cavernosa and tunica albuginea. Such treatment is expected to result in permanently improved erectile function for many (although not all) ED patients, and to finally offer a non-surgical option or as a co-adjuvant for the treatment of PD. Human studies have supported some of these preclinical findings and their interpretation, but their discussion is beyond the scope of this paper, although some reviews from other authors have stated the relevance of a primarily anti-fibrotic PDE5i strategy for the treatment of PD, coupled with its smooth muscle protection action for the treatment of CVOD, and lower urogenital tract symptoms (LUTS), mainly in the context of daily PDE5i [22–28]. We look forward to seeing what new advances of PDE5i preclinical and clinical research will bring for their use as an anti-fibrotic and smooth muscle protective therapeutic approach, often coupled to stem cell treatment, not just for these conditions, but in other chronic serious fibrotic processes in various urological and non-urological conditions [17, 29–33].

Compliance with ethical standards

Conflict of interest Dr. Gonzalez-Cadavid and Dr. Rajfer have a financial interest in a related U.S. Patent and its continuation.

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