Sildenafil Promotes Smooth Muscle Preservation and Ameliorates Fibrosis Through Modulation of Extracellular Matrix and Tissue Growth Factor Gene Expression After Bilateral Cavernosal Nerve Resection in the Rat

Fara Sirad, BS,* Su Hlaing, BS,* Istvan Kovanecz, PhD,†§ Jorge N. Artaza, MS, PhD,*‡
Leah A. Garcia, BS,* Jacob Rajfer, MD,†§ and Monica G. Ferrini, MS, PhD*‡

*Department of Internal Medicine, Charles R. Drew University of Medicine & Science, Los Angeles, CA, USA; †Division of Urology, Department of Surgery, Harbor-UCLA Medical Center, and Los Angeles Biomedical Research Institute (LABiomed), Torrance, CA, USA; ‡Department of Medicine David Geffen School of Medicine at UCLA, Los Angeles, CA, USA; §Department of Urology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

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ABSTRACT

Introduction. It has been shown that phosphodiesterase type 5 (PDE5) inhibitors preserve smooth muscle (SM) content and ameliorate the fibrotic degeneration normally seen in the corpora cavernosa after bilateral cavernosal nerve resection (BCNR). However, the downstream mechanisms by which these drugs protect the corpora cavernosa remain poorly understood.

Aim. To provide insight into the mechanism, we aimed to determine the gene expression profile of angiogenesis-related pathways within the penile tissue after BCNR with or without continuous sildenafil (SIL) treatment.

Methods. Five-month-old Fisher rats were subjected to BCNR or sham operation and treated with or without SIL (20 mg/kg/BW drinking water) for 3 days or 45 days (N = 8 rats per group). Total RNAs isolated from the denuded penile shaft and prostate were subjected to reverse transcription and to angiogenesis real-time-polymerase chain reaction arrays (84 genes). Changes in protein expression of selected genes such as epiregulin (EREG) and connective tissue growth factor (CTGF) were corroborated by Western blot and immunohistochemistry.

Main Outcomes Measures. Genes modulated by BCNR and SIL treatment.

Results. A decreased expression of genes related to SM growth factors such as EREG, platelet-derived growth factor (PDGF), extracellular matrix regulators such as metalloproteinases 3 and 9, endothelial growth factors, together with an upregulation of pro-fibrotic genes such as CTGF and transforming growth factor beta 2 were found at both time points after BCNR. SIL treatment reversed this process by upregulating endothelial and SM growth factors and downregulating pro-fibrotic factors. SIL did not affect the expression of EREG, VEGF, and PDGF in the ventral prostate of BCNR animals

Conclusions. SIL treatment after BCNR activates genes related to SM preservation and downregulates genes related to fibrosis in the corpora cavernosa. These results provide a mechanistic justification for the use of SIL and other PDE5 inhibitors as protective therapy against corporal SM loss and fibrosis after radical prostatectomy. **Sirad F, Hlaing S, Kovanecz I, Artaza JN, Garcia LA, Rajfer J, and Ferrini MG. Sildenafil promotes smooth muscle preservation and ameliorates fibrosis through modulation of extracellular matrix and tissue growth factor gene expression after bilateral cavernosal nerve resection in the rat. J Sex Med 2011;8:1048–1060.**

Key Words. Sildenafil; Angiogenesis; Fibrosis; Bilateral Cavernosal Nerve Resection; Epiregulin, Smooth Muscle

Introduction

Erectile dysfunction (ED) associated with radical prostatectomy (RP) is a common complication after surgery due to intra-operative injury to the cavernosal nerves [1,2]. Although nerve-sparing RP (NSRP) has significantly decreased the rates of postoperative ED [2–4], novel approaches for preserving both the normal architecture of the corporal tissue as well as its function continue to undergo investigation because of the still unacceptable high rates of ED with NSRP.

Cavernosal nerve damage ultimately leads to apoptosis of the corporal smooth muscle (SM) cells as well as fibrosis of the corporal tissue [5,6]. This condition leads to the development of cavernosal veno-occlusive dysfunction (CVOD) [6] which is sometimes referred to as venous leakage. In this condition, blood entering the corporal tissue during an erectile event fails to remain within the sinusoids mainly because the remaining SM within the cavernosa is unable to relax sufficiently enough to achieve an intracorporeal pressure high enough to occlude the veins egressing the corpora. This alteration in the corporal histology is initiated immediately after the nerve injury occurs and it is assumed that a certain threshold of SM impairment/apoptosis has to be reached before a functional impairment, i.e., ED due to CVOD become manifest [7]. Therefore, in this setting, therapies to protect corporal SM viability either by reducing its apoptosis and/or sustaining its proliferation should be initiated as early as possible after the injury.

It has been previously demonstrated in rats that long-term treatment with the phosphodiesterase type 5 (PDE5) inhibitors such as vardenafil [6], sildenafil (SIL) [8], and tadalafil [9] preserves corporal SM and ameliorates fibrotic degeneration normally seen after bilateral cavernosal nerve resection (BCNR). Anecdotally, acute SIL treatment is able to downregulate penile hypoxia markers [10]. There is still ongoing debate as to the exact role that PDE5 inhibitors play in the post-RP setting [11]. One school of thought states that these PDE5 inhibitors increase "oxygenation" to the penile tissues [10,12–14], while the other school of thought adheres to the concept that these are simply anti-fibrotic compounds which primarily inhibit the oxidative stress to the cavernosal tissue induced by the neurotomy [6–9,15–18].

Although animal studies support the concept of so-called corporal preservation post-prostatectomy, in the clinical setting, there is no consensus regarding its benefit, mainly because all the studies performed so far have marked differences in their design that comparisons between studies cannot be made. For example, the study of Schwartz et al. [19] reported on 40 patients who had undergone RP and were treated with one of two doses of SIL started when the catheter was removed and given every other day for 6 months and who had a cavernosal biopsy both prior to and at the end of the study period. The results demonstrated not only preservation of corporal histology and absence of fibrosis, but in the higher-dose group, there was a substantial increased content of corporal SM cells. Padma Nathan and et al. [20] found in a randomized, double-blind, placebo-controlled study of postoperative nightly SIL started 4 weeks after the NSRP and administered nightly for 36 weeks a fourfold increase in the return of normal spontaneous erections when compared to the control group, although in the control group, only 8% had normal erections at the end of the study period. More recently, Montorsi et al. [21], in the triple arm REINVENT study where drug treatment was initiated 14 days after the NSRP and given for 9 months, reported that vardenafil was more efficacious in preserving erectile function when given as an on-demand rather than as a nightly dose, although in the final open label phase of the study, there was no difference in terms of recovery of erections as determined by an on-demand vardenafil trial. In fact, both the daily and on-demand treatment for 9 months was no different than the placebo group

From these data, it is not surprising that penile rehabilitation or corporal preservation after RP remains controversial and more research is required in order to establish, at least with PDE5 inhibitors, when to start treatment post-RP, as well as the dose and the appropriate outcome metric to determine efficacy [22,23].

To date, little is known about the mechanism of action by which long-term PDE5 inhibitors would have a beneficial effect on the corporal tissue. While it is known that elevated levels of cyclic guanosine monophosphate may inhibit SM apoptosis and/or inhibit collagen deposition, the downstream pathways involved in this process are poorly understood. Angiogenesis, the formation of thin-walled endothelium-lined structures with muscular SM wall, plays an important role during the adult life span as "repair mechanism" of damaged tissues [24]. Emerging evidence has demonstrated that SIL can induce angiogenesis after