The Functional and Structural Consequences of Cavernous Nerve Injury are Ameliorated by Sildenafil Citrate

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DOI: 10.1111/j.1743-6109.2008.00794.x

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ABSTRACT

Introduction. Radical prostatectomy (RP) is associated with erectile dysfunction (ED). A single, placebo-controlled, human study has assessed the effects of regular sildenafil use after RP and demonstrated an increased chance of preservation of preoperative erectile function.

Aim. This study was undertaken to define the effects of such a regimen in an animal model.

Methods. Using the cavernous nerve (CN) crush injury model, animals were divided into a number of groups: no CN injury (sham), bilateral CN injury exposed to either no sildenafil (control) or sildenafil at two doses (10 and 20 mg/kg) subcutaneously daily for three different durations (3, 10, 28 days).

Main Outcome Measures. At these time points, CN electrical stimulation was used to assess erectile function by mean intracavernosal pressure (ICP)/mean arterial pressure (MAP) ratio. For the structural analyses, whole rat penes were harvested. Staining for Masson's trichrome was utilized to calculate the smooth muscle-collagen ratio. Immunohistochemical antibody staining was performed for endothelial (CD31 and eNOS) and neural (GAP43, NGF, and nNOS) factors and immunoblotting was performed to analyze the AKT/eNOS pathway. Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) assay was used for the assessment of apoptotic indices and the CN architecture was evaluated by transmission electron microscopy (TEM).

Results. Erectile function was improved with sildenafil in a time- and dose-dependent fashion with maximization of erectile function recovery occurring with daily 20 mg/kg at the 28-day time point. Sildenafil use resulted in smooth muscle-collagen ratio protection and CD31 and eNOS expression preservation. Sildenafil reduced apoptotic indices significantly compared with control. Animals exposed to sildenafil had increased phosphorylation of akt and eNOS. Tem demonstrated distinct differences in architecture between control and sildenafil groups toward an increased amount of myelinized nerve fibers.

Conclusions. Sildenafil use in the CN crush injury model preserves erectile function that appears to be mediated predominantly through preservation of smooth muscle content and endothelial function as well as through reduction in apoptosis. **Mulhall JP, Müller A, Donohue JF, Mullerad M, Kobylarz K, Paduch DA, Tal R, Li PS, Cohen-Gould L, and Scardino PT. The functional and structural consequences of cavernous nerve injury are ameliorated by sildenafil citrate. J Sex Med 2008;5:1126–1136.**

Key Words. Erectile Dysfunction; Cavernous Nerve; Sildenafil Citrate; Apoptosis

Introduction

Approximately 50,000 radical prostatectomies (RP) are performed each year in the United States for the treatment of prostate cancer. This operation is associated with at least transient erectile dysfunction (ED), with ED rates ranging from 20% to 90% depending upon the study reviewed [1–4]. It is postulated that the development of post-RP ED is due predominantly to a combination of temporary erectile (cavernous) nerve injury and damage to the erectile tissue secondary to

neuropraxia and potentially absence of cavernosal oxygenation [5].

A single human, randomized, placebo-controlled study has been conducted examining the role of nightly sildenafil for a 36-week time period following RP [6]. This study demonstrated the ability of this regimen to increase the rate of preservation of preoperative erectile function at approximately 1 year postoperatively in the sildenafil arm compared with the placebo arm [6]. In another noncontrolled study, Schwartz et al. demonstrated that regular use of sildenafil post-RP preserved cavernosal smooth muscle content [7].

The presumed mechanism of this apparent protective effect of sildenafil was originally believed to be cavernosal oxygenation related to penile erection; however, more recently, some have postulated that sildenafil may have an endothelial or neuroprotective effect [8]. The rat cavernous nerve (CN) injury model is believed to simulate the neural injury that occurs during RP and is designed as a model to study the mechanisms of ED post-RP as well as to explore ED-minimizing strategies [9]. This study was undertaken to generate animal data in support of human studies and to explore the mechanisms by which sildenafil may preserve erectile function in this animal model.

Methods

Animal Groupings and Sildenafil Administration

Sprague-Dawley rats, initially weighing 250–300 g, were randomly divided into four groups: (i) sham (no CN crush, no sildenafil); (ii) control (C; bilateral CN crush, no sildenafil); and two treatment groups (bilateral CN crush, sildenafil sc): (iii) sildenafil 10 mg/kg (S10); and (iv) sildenafil 20 mg/kg (S20) subcutaneously daily commencing day of CN crush until 24 hours prior to sacrifice. Within each of the four groups, three time subgroups were analyzed, 3 days, 10 days, and 28 days after CN crush. Ten animals were analyzed for the following groups: sham, C28, S20-28; and five animals in each additional subgroup for a total number of 65 animals. The animals were cared for and housed under strict guidelines established by the Cornell University Institutional Animal Care and Use Committee guidelines.

CN Injury

For the initial surgery, the animals were anesthetized using 4% isoflurane and placed in the supine position. Through a lower midline incision, the major pelvic ganglion (MPG) lying on the dorsal prostate and the CN emanating form the ganglion were identified using a Zeiss operating microscope. For the CN crush injury, 5 mm distal to the MPG, a #7 Dumont hemostat was applied to the CN for 30 seconds, removed for 30 seconds and then reapplied for a further 30 seconds.

CN Stimulation

At either 3, 10 or 28 days after CN crush, the animals were anesthetized for the second, nonsurvival surgery. The left internal carotid artery was cannulated with heparinized polyethylene-50 tubing, connected to a pressure transducer and an amplifier unit (Harvard Apparatus, Holliston, MA, USA) recording the mean arterial pressure (MAP). The amplifier was attached to a data acquisition module (DI-190, Dataq Instruments, Akron, OH, USA). Measurement of the intracavernosal pressure (ICP) was achieved by inserting a 24-gauge needle into the corporal body. After CN identification, a stainless steel bipolar electrode with parallel hooks (1 mm apart) was placed around the nerve, just distal to the ganglion but proximal to where the nerve had been crushed. The electrode cable was linked to a Grass S48 stimulator (Quincy, MA, USA), and stimulated (parameters of 1.5 mA, 20 Hz, pulse width 5 milliseconds, at 7.5 volts) for 60 seconds each. Both nerves were stimulated and the maximal ICP generated with the corresponding MAP (ICP/MAP ratio) was recorded and presented as percentage.

Tissue Harvesting

Structural analyses were performed only on the 28-day group specimens. At the completion of CN stimulation and icp/map ratio recording, the penis was detached from its attachments to the ischiopubic rami and transected. On a side table, using a Zeiss operating microscope, the penis was microdissected so that all extra-tunical tissue was removed. The whole penis was divided into segments for embedding in paraffin (for terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) and immunohistochemistry) and for snap freezing (for immunohistochemistry and molecular analyses).

Histopathology

Following routine dehydration and paraffin embedding, tissue samples were cut into 5–7 μm sections from the mid-shaft of the penis mounted on slides and dried. Then the tissue slides, show-