THE JOURNAL OF
SEXUAL MEDICINE

Off-Target Effect of Sildenafil on Postsurgical Erectile Dysfunction: Alternate Pathways and Localized Delivery System

Amirali Salmasi, MD,[1,2,*] Geun Taek Lee, PhD,[1,*] Neal Patel, MD,[1,2] Ritu Goyal, PhD,[3] Michael Dinizo, MD,[1,2] Young Suk Kwon, MD,[1] Part K. Modi, MD,[1,2] Izak Faiena, MD,[1,2] Nara Lee, MD,[4] Johanna L. Hannan, PhD,[5] Joachim Kohn, PhD,[3] and Isaac Yi Kim, MD, PhD[1,2]

ABSTRACT

Introduction: There is no consensus on the best oral phosphodiesterase type 5 inhibitor (PDE5I) for patients undergoing penile rehabilitation after surgical nerve injury.

Aim: To determine the mechanism of PDE5I on cultured neuronal cells and the effectiveness of local drug delivery using nanospheres (NSPs) to sites of nerve injury in a rat model of bilateral cavernous nerve injury (BCNI).

Methods: The effects of sildenafil, tadalafil, and vardenafil on cyclic adenosine monophosphate, cyclic guanosine monophosphate, and cell survival after exposure to hypoxia and H_2O_2 were measured in PC12, SH-SY5Y, and NTERA-2 (NT2) cell cultures. The effects of phosphodiesterase type 4 inhibitor (PDE4I) and PDE5I on neuronal cell survival were evaluated. Male rats underwent BCNI and were untreated (BCNI), immediately treated with application of empty NSPs (BCNI + NSP), NSPs containing sildenafil (Sild + NSP), or NSPs containing rolipram (Rol + NSP).

Main Outcome Measures: Viability of neuronal cells was measured. Intracavernous pressure changes after cavernous nerve electrostimulation and expression of neurofilament, nitric oxide synthase, and actin in mid-shaft of penis were analyzed 14 days after injury.

Results: Sildenafil and rolipram significantly decreased cell death after exposure to H_2O_2 and hypoxia in PC12, SH-SY5Y, and NT2 cells. PC12 cells did not express PDE5 and knockdown of PDE4 significantly increased cell viability in PC12, SH-SY5Y, and NT2 cells exposed to hypoxia. The ratio of intracavernous pressure to mean arterial pressure and expression of penile neurofilament, nitric oxide synthase, and actin were significantly higher in the Sild + NSP and Rol + NSP groups than in the BCNI and BCNI + NSP groups. Limitations included analysis in only two PDE families using only a single dose.

Conclusion: Sildenafil showed the most profound neuroprotective effect compared with tadalafil and vardenafil. Sildenafil- or rolipram-loaded NSP delivery to the site of nerve injury prevented erectile dysfunction and led to increased neurofilament, nitric oxide synthase, smooth muscle content in rat penile tissue after BCNI.

J Sex Med 2016;■:1–10.

Key Words: Phosphodiesterase Inhibitors; Cavernous Nerve Injury; Iatrogenic Erectile Dysfunction; Penile Rehabilitation; Radical Prostatectomy

Received March 16, 2016. Accepted October 12, 2016.

[1]Section of Urologic Oncology, Rutgers Cancer Institute of New Jersey, New Brunswick, NJ, USA;

[2]Division of Urology, Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, USA;

[3]New Jersey Center for Biomaterials, Rutgers University, Piscataway, NJ, USA;

[4]Department of Medicine, Rutgers Cancer Institute of New Jersey and Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ, USA;

[5]Department of Physiology, Brody School of Medicine, East Carolina University, Greenville, NC, USA

*Equivalent contribution.

http://dx.doi.org/10.1016/j.jsxm.2016.10.003

INTRODUCTION

The risk of erectile dysfunction (ED) after radical prostatectomy (RP) is 20% to 80%.[1,2] The etiology of post-RP ED is believed to be multifactorial and involves a combination of ischemic, mechanical, thermal, and inflammatory injuries to the neurovascular bundles.[3,4] Various treatment modalities for post-prostatectomy ED, including pharmacologic agents and devices, have been studied, but no standard treatment algorithm or guideline for effective penile rehabilitation has been developed. A promising and widely evaluated intervention is the use of phosphodiesterase type 5 inhibitors (PDE5Is).[4,5]

Animal studies have shown that PDE5I alleviates ED and reverses adverse structural changes seen after bilateral cavernous

nerve injury (BCNI).[6–9] These previous studies reported that the main mechanisms of PDE5I include prevention of oxidative-associated tissue damage and preservation of penile smooth muscle and endothelial integrity. In addition, much attention has been given to the direct effect of PDE5I on damaged nerves. Hlaing et al[10] reported that oral sildenafil after cavernous nerve resection in rats increases neurotrophic factors and has a neuroregenerative effect on major pelvic ganglia. Furthermore, sildenafil has been shown to protect the central nervous system against stroke or chemical hypoxia in animal models by increasing blood flow and minimizing hypoxia.[11–14] Although the precise mechanism underlying the neuroprotective effect of PDE5I remains unclear, cyclic guanosine monophosphate (cGMP) and nitric oxide (NO) have been implicated as key mediators.[11,15]

Orally administered PDE5I treatments for post-RP ED often require long-term daily use and can cause dose-related side effects, such as headache, dizziness, flushing, dyspepsia, myalgia, and nasal congestion. Local delivery of these drugs might be more favorable than systemic (oral) delivery for the following reasons: relative ease of laparoscopic administration; high bioavailability by localized direct delivery of the therapy to the wound site; and decreased occurrence of unwanted systemic side effects. The use of nanospheres (NSPs) represents an innovative approach to increase the bioavailability, solubility, circulation time, and resistance to metabolic degradation of hydrophobic drugs. These NSPs are synthesized from a family of fully degradable, ABA-type triblock copolymers made of poly(ethylene glycol), oligomers of desamino-tyrosyl-tyrosine esters, and suberic acid[16] and spontaneously self-assemble in aqueous media. Several preclinical studies have reported on the non-toxicity of these NSPs in vitro[16] and in vivo[17] and on their ability to deliver hydrophobic drugs.[16–18]

In the present study, we investigated the mechanism underlying the neuroprotective effect and the impact of local delivery of PDEIs, using NSPs on erectile function (EF) after BCNI, in an effort to further understand the role of PDE5Is in the framework of post-RP ED.

METHODS

Chemicals and Reagents

Sildenafil citrate, tadalafil, vardenafil, and rolipram (Selleck Chemicals, Houston, TX, USA) were dissolved in dimethyl sulfoxide and diluted with complete media. H_2O_2 was diluted in complete media.

Cell Culture and Treatment

The human neuroblastoma cell line, SH-SY5Y (ATCC, Manassas, VA, USA), was maintained in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). We evaluated differentiated and undifferentiated SH-SY5Y cell lines in this study. To induce differentiation, we treated SH-SY5Y cells with DMEM containing 3% FBS and all-trans retinoic acid 10 μmol/L for 72 hours. PC12 (ATCC), a rat pheochromocytoma cell line, was cultured in DMEM containing 10% horse serum and 5% FBS. Human pluripotent NTERA-2 (NT2) cell culture (ATCC) was cultured in 10% FBS and 1% penicillin and streptomycin with DMEM under an atmosphere of 5% CO_2 at 37°C. Generation of NT2 neurons was performed using a differentiation protocol in free-floating aggregates. Briefly, NT2 cells were seeded in bacteriological-grade Petri dishes at a density of 5×10^6 cells. On the first day, culture medium 10 mL was added to each Petri dish. On the next days, all-trans retinoic acid 10 μmol/L was added. The media were changed every 2 to 3 days. After 8 days, the cells from Petri dish were transferred and seeded into100-mm cell culture dishes and cultured for another 8 days in retinoic acid medium. The BD GasPak EZ Anaerobe Pouch System (Becton Dickinson, Franklin Lakes, NJ, USA) was used for the hypoxia assay. This system produces an anaerobic atmosphere (oxygen concentration $< 0.1\%$) within 2.5 hours with greater than or equal to 10% carbon dioxide within 24 hours. Cells were cultured under hypoxia for 24 hours. For the H_2O_2 toxicity assay, NT2 cells, SH-SY5Y, and PC12 cells were incubated with the indicated concentration of H_2O_2 for 24 hours. Cells were incubated with different concentrations of sildenafil, tadalafil, vardenafil, and rolipram for 30 minutes before H_2O_2 administration. Cell density and viability were determined using the Vi-Cell Viability Analyzer (Beckman Coulter, Fullerton, CA, USA).

Cyclic Adenosine Monophosphate and cGMP Assay

Cells were seeded in 24-well plates and exposed to 24 hours of hypoxia after incubation with the indicated concentrations of sildenafil, tadalafil, or vardenafil. Extracellular levels of cyclic adenosine monophosphate (cAMP) and cGMP were measured with immunoassay kits (Abcam, Cambridge, MA, USA).

RNA Isolation, Reverse Transcription, and Polymerase Chain Reaction

Total RNA was isolated using TRIzol reagent (Invitrogen, Waltham, MA, USA). Isolated RNA was reverse transcribed (ImProm-II Reverse Transcription System; Promega, Madison WI, USA) and cDNA 1 μg was used for polymerase chain reaction (PCR) over 35 cycles. Rat and human PDE primers were used (Table 1). β-Actin mRNA was used as a control. We also purified RNA from paraffin-embedded tissue using a Paraffin Embedded Tissue RNA Isolation Kit from Thermo Fisher (Waltham, MA, USA).

Transient Transfection

For transfection, 50,000 cells were plated onto six-well plates. PDE4a, PDE4b, PDE4d, or PDE5a small interfering RNA (Qiagen, Valencia, CA, USA) was transfected using a PC12 transfection reagent (Altogen Biosystem, Las Vegas, NV, USA)