Chronic Administration of Udenafil, A Selective Phosphodiesterase Type 5 Inhibitor, Promotes Erectile Function Recovery in an Animal Model of Bilateral Cavernous Nerve Crush Injury

Chan-Ho Lee, RPh, PhD,* Hae-Sun Kim, MS,* Moon-Jung Goo, DVM, MS,* Kyung-Koo Kang, DVM, PhD,* Byoung-Ok Ahn, DVM, PhD,* Soon Hoe Kim, RPh, PhD,* and Dae-Yul Yang, MD, PhD†

*Research Laboratory, Dong-A Pharmaceutical Company, Giheung, Yongin, Gyeonggi, Korea; †Department of Urology, Kangdong Sacred Heart Hospital, Hallym University, Seoul, Korea

DOI: 10.1111/j.1743-6109.2011.02228.x

ABSTRACT

Introduction. Preservation of the cavernous nerves (CNs) during radical prostatectomy is crucial for the patient’s erectile function. Despite advances in operative technique, the majority of men report compromised erectile function postprostatectomy or complete loss of potency due to CN trauma even with nerve-sparing modifications.

Aim. This study was designed to investigate whether repeated dosing of udenafil, a phosphodiesterase type 5 inhibitor, helps to improve erectile function after CN injury.

Methods. Using the CN crush injury model, 8-week-old male Sprague Dawley rats were divided into the following groups; sham-operated group, bilateral CN crush injury exposed to either no udenafil (vehicle) or udenafil (5, 20 mg/kg) daily for two different durations (4 and 8 weeks, p.o.).

Main Outcome Measures. At both time points, CN electrical stimulation was used to assess erectile function by measuring the intracavernous pressure. The expressions of hypoxia-inducible factor 1-alpha (HIF-1α), transforming growth factor-beta (TGF-β1), nerve growth factor (NGF), endothelin B receptor (ETB), endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and sonic hedgehog homolog (SHH) in penile tissue were examined. Immunohistochemical antibody staining was performed for NGF, eNOS, nNOS, CD31, and alpha-smooth muscle actin (α-SMA). Additionally, terminal deoxynucleotidyl transferase-mediated nick-end labeling assay was performed to quantify apoptosis and the tissue slides were stained for Masson’s trichrome to assess the smooth muscle/collagen ratio.

Results. Udenafil improved erectile function in a dose- and time-dependent manner with the maximum erectile function recovery achieved by 20 mg/kg udenafil at an 8-week time point. CN injury increased the expression of HIF-1α, TGF-β1, NGF, and ETB, however, decreased the expression of eNOS, nNOS, and SHH. Udenafil significantly suppressed these alterations. The results from the histological analyses show that udenafil markedly reduces apoptosis induced by CN injury and augments the smooth muscle/collagen ratio.


Key Words. Apoptosis; Cavernous Nerve Crush Injury; Erectile Dysfunction; Sonic Hedgehog; Udenafil
Introduction

Radical prostatectomy is a commonly performed procedure for clinically localized prostate cancer and despite the advent of advanced techniques for the management of pelvic malignancies, a substantial proportion of men report compromised erectile function postoperatively [1,2]. The rat model of cavernous nerve (CN) crush injury (CNCI) demonstrates various sequelae, such as apoptosis, upregulation of fibrogenic cytokines, smooth muscle fibrosis, and reduction in nitric oxide synthase [3], and is designed to investigate therapeutic strategies minimizing erectile dysfunction as well as to study the mechanisms of prostatectomy-induced erectile dysfunction [4]. Recent animal and human studies, such as the Recovery of Erections: Intervention with Vardenafil Early Nightly Therapy (REINVENT) study, have reported the potential therapeutic role of phosphodiesterase type 5 (PDE5) inhibition in erectile dysfunction following nerve-sparing radical prostatectomy [5–7].

Udenafil (Zydena®) is a selective PDE5 inhibitor developed for the treatment of erectile dysfunction [8]. Recent studies have reported the safety and efficacy of udenafil in men with erectile dysfunction [9,10]. In preclinical studies, udenafil improved penile responses in normal, diabetic, and spinal cord-injured animals via the selective inhibition of PDE5 [11–14]. This study was performed to gain novel insights into the mechanism of action by which chronic treatment of udenafil ameliorates the erectile dysfunction induced by CNCI in rats, and as a result, lend support for future clinical investigation. In this study, we investigated the effect of udenafil on erectile function, gene and protein expression, smooth muscle fibrosis, and apoptosis in the rat model of CNCI model. This study also reports, for the first time, the effect of PDE5 inhibition on the expression of sonic hedgehog (SHH), which has been recently identified as an essential regulator of penile smooth muscle, apoptosis, and erectile function [15,16].

Methods

Animals and Udenafil Administration

Eight-week-old male Sprague Dawley rats (Charles River Laboratories Inc., Yokohama, Japan), 10 to 12 animals per group, were randomly divided into the following groups: (i) sham-operated (Sham); (ii) CNCI; (iii) 5 mg/kg udenafil treatment group (CNCI + 5 U); and (iv) 20 mg/kg udenafil treatment group (CNCI + 20 U). The experiment was carried out with two sets of the four experimental groups mentioned above. Data from the animals in the first set were collected after 4-week treatment, and data from the second set of animals were collected after 8-week treatment. After CN injury was applied by the bilateral CNCI technique, animals in the udenafil treatment group received daily oral administrations of udenafil (5 or 20 mg/kg, CAS No.; 268203-93-6, 5-[2-propoxy-5-(1-methyl-2-pyrrolidinylethylaminoisulfonyl) phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one, Dong-A Pharmaceutical Company, Seoul, Korea), by gavage, commencing day of CNCI until 24 hours prior to evaluation. Other animals received vehicle (Titrisol buffer solution, citrate sodium hydroxide buffer, pH 5.0, Merck, Darmstadt, Germany) treatments. The treatments were well tolerated by all animals. Animals were cared for and housed under standard laboratory conditions, and food and ultraviolet-sterilized tap water were provided ad libitum. The animal experiments of this study were approved by the Institutional Animal Care and Use Committee of Dong-A Pharmaceutical Company and performed in accordance with the “Principles of Laboratory Animal Care” established by the National Institutes of Health.

Bilateral CNCI Model

For the CNCI surgery, animals were anesthetized with an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg), and kept isothermic using a heating pad. Through an abdominal incision, the prostate was exposed, and the major pelvic ganglion lying on the dorsal prostate and the CN emanating from the ganglion were identified. For the CNCI, 5 mm distal to the major pelvic ganglion, the tips of an ultra fine curved hemostat were applied to the CN for 30 seconds, removed for 30 seconds, and then reapplied for a further 30 seconds, at a constant “two-click” pressure. The injury was applied bilaterally and the abdominal incision was closed layer by layer. Laparotomy only was performed in the sham group.

Electrical Stimulation of the CN

In order to evaluate the erectile function, 24 hours after the final vehicle or drug administration, the intracavernous pressure (ICP) and mean arterial pressure (MAP) were measured in anesthetized animals in a blinded fashion with the person measuring the ICP and MAP unaware of the treat-
The left carotid artery was exposed and cannulated with a PE-50 tube filled with 50 IU heparinized saline to monitor the MAP. ICP was monitored by inserting a 23 G needle, connected to polyethylene tubing filled with heparinized saline, into the corpus cavernosum after the penis was denuded of skin and fascia. After CN identification, a bipolar electrode with parallel hooks 1 mm apart was placed around the nerve, just distal to the major pelvic ganglion but proximal to where the nerve had been crushed. Erections were achieved by CN electrical stimulation (1.5 mA, 20 Hz, with square-wave duration of 5 ms, at 7.5 V) for 60 seconds. MAP and ICP were recorded on a polygraph, and data acquisition and calculation of the derived parameters were performed using a signal processor. Erectile function was represented by the maximum ICP/MAP (%).

**RNA Isolation and Reverse Transcription-Polymerase Chain Reaction (RT-PCR)**

Freshly dissected corpus cavernosum samples from 4- or 8-week-treated rats were subjected to RT-PCR amplification for hypoxia-inducible factor (HIF)-1α, transforming growth factor (TGF)-β1, nerve growth factor (NGF), endothelin B receptor (ETb), endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and SHH mRNA. Total RNA samples were isolated using the Trizol (Invitrogen, Carlsbad, CA, USA) method and were quantified by the following procedures. The primer sequences used are shown in Table 1. Isolated RNA samples were reverse-transcribed to first strand cDNA at 48°C for 45 minutes, and RNA/cDNA hybrids were denatured by 2-minute incubation at 94°C. All procedures were carried out using the Access RT-PCR system (Promega, Madison, WI, USA). One-percent agarose gel electrophoresis and ethidium bromide staining were used to verify the PCR products.

**Protein Extraction and Western Blot Analysis**

One-fourth of each penile tissue was homogenized separately in RIPA buffer with protease inhibitor (Complete Protease Inhibitor Cocktail Tablets, Roche Applied Science, Indianapolis, IN, USA). The homogenate was centrifuged at 14,000 × g for 10 minutes, and the supernatants were recovered. The protein concentration was measured using the BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA) and then calculated with SoftMax Pro software version 5.2 (Molecular Devices, Sunnyvale, CA, USA). After denaturation of the total protein at 95°C for 5 minutes, protein samples (30–45 μg of protein/lane) were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on –4–12% gradient gels (Invitrogen). The resolved proteins were transferred onto a 0.45-μm nitrocellulose membrane by semidy electroblotting for 1 hour, and soaked in 5% nonfat dry milk in Tris-buffered saline containing 0.05% Tween-20 for 1 hour at room temperature. The membrane was incubated with 1:1,000 nNOS, eNOS mouse monoclonal antibodies (BD Transduction Laboratories, San Jose, CA, USA), SHH (C9C5) rabbit monoclonal antibody (Cell Signaling Technology, Beverly, MA, USA) or 1:5,000 β-actin mouse monoclonal antibody (Abcam, Cambridge, MA, USA) overnight at 4°C. Membranes were washed with TBS-T three times for 30 minutes and then incubated with 1:8,000 anti-mouse and 1:5,000 anti-rabbit secondary antibodies for 1 hour at room temperature. Protein bands were visualized using chemiluminescence (ECL plus Western Blotting Detection Reagents, Amersham-Pharmacia Biotech, Piscataway, NJ, USA) according to the manufacturer’s instruc-

---

**Table 1**  **Primer sequences used for PCR amplification**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5′→3′)</th>
<th>Reverse primer (5′→3′)</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIF-1α</td>
<td>GTCAGGAGGATGCAGCACGATCTCCTG</td>
<td>GGTGATGCAGATGCAGTCGCAAGGC</td>
<td>1302</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>CCGGAAAGGGGGCTCAACACC</td>
<td>GTGTGACACTGCCTCACCT</td>
<td>352</td>
</tr>
<tr>
<td>NGF</td>
<td>TGGACCAAGCTCACAACGCTG</td>
<td>GTCTATCTCACAACCCACACA</td>
<td>531</td>
</tr>
<tr>
<td>ETb</td>
<td>TCTAACAAGGAGGATCTTCTG</td>
<td>AGTGTGAAAGTGAAGGAC</td>
<td>473</td>
</tr>
<tr>
<td>eNOS</td>
<td>CAGGCTGTCGCTGAACTT</td>
<td>GATCTGCTACGCTGACCTT</td>
<td>473</td>
</tr>
<tr>
<td>nNOS</td>
<td>CTTCCTGAGATGTTCTG</td>
<td>TGGACCGGTGAGGTGCCCGCC</td>
<td>473</td>
</tr>
<tr>
<td>SHH</td>
<td>CTGCCAGGATGTTCTG</td>
<td>TGCATTGGGGGTGCACCT</td>
<td>232</td>
</tr>
<tr>
<td>β-Actin</td>
<td>TCTAACAATGAGCGGTGTTG</td>
<td>AGGTGAGCATGGAGGAC</td>
<td>259</td>
</tr>
</tbody>
</table>

HIF-1α = hypoxia-inducible factor-1 alpha; TGF-β1 = transforming growth factor-beta 1; NGF = nerve growth factor; ETb = endothelin ETb receptor; eNOS = endothelial nitric oxide synthase; nNOS = neuronal nitric oxide synthase; SHH = sonic hedgehog.
tions. Bands imaged by a Molecular Imager ChemiDoc XRS System (Bio-Rad Laboratories, Hercules, CA, USA) were quantified with Quantity One software (Bio-Rad Laboratories). Quantification of the bands was performed by determining the ratio of the density of eNOS, nNOS, and SHH divided by β-actin.

**Immunohistochemistry**

Sectioned corporal tissue of the rat penis was assessed by the routine immunohistochemistry method using antibodies for NGF (1:400), eNOS (1:70), nNOS (1:100), CD31 (1:30), and α-smooth muscle actin (SMA; 1:100) (Abcam). The tissue samples were immunostained overnight at 4°C with antibodies diluted in antibody diluent. Immunoreactive materials were visualized with avidin-biotin-peroxidase complex solution using a Vectastain ABC Kit (Vector Laboratories, Burlingame, CA, USA) with 3,3-diaminobenzidine (Zymed Laboratories, San Francisco, CA, USA). In order to quantify apoptosis in the penile cavernous tissue, terminal deoxynucleotidyl transferase-mediated nick-end labeling staining was performed using a commercial apoptosis detection kit (Roche Diagnostica GmbH, Penzberg, Germany) according to the manufacturer’s instructions. For image analysis, five randomly selected fields per animal were photographed and recorded at ×400 magnification. Images were analyzed with Image-Pro Plus 4.1 software (Media Cybernetics, Bethesda, MD, USA).

**Histopathology**

Corporal tissues from each group were fixed in 10% buffered formalin for morphological analysis. Tissue samples were embedded in paraffin and cut into 5–7 μm sections from the mid-shaft of the penis. The tissue slides, showing the cross-section of the corpora cavernosa, were deparaffinized, rehydrated, and stained for Masson’s trichrome to assess the smooth muscle/collagen ratio, utilizing Image Pro Plus 4.1 software (Media Cybernetics). In each group, a total of 15 sections were reviewed for histomorphometry.

**Statistical Analysis**

The overall significance of the experimental results was analyzed by analysis of variance test. The differences between the groups were considered significant at P < 0.05 with the appropriate Bonferroni correction made for multiple comparisons. The results are presented as the mean ± standard deviation unless otherwise stated.

**Results**

**Erection Physiology Studies**

At 4 and 8 weeks after the CNCI surgery, erectile function was significantly diminished in vehicle-treated animals compared with sham-operated animals (P < 0.05) (Figure 1). However, daily administration of udenafil (5 or 20 mg/kg) resulted in an improvement in the mean ICP/MAP ratio at all time points, compared with the animals in the CNCI group (P < 0.05). As shown in Figure 1, following 4 and 8 weeks of daily udenafil treatment (5 mg/kg), the mean ICP/MAP ratios were 53.9 ± 10.9 and 64.8 ± 10.4, respectively. The
ICP/MAP ratios were 56.3 ± 9.4 and 76.8 ± 11.8 after 20 mg/kg udenafil treatment for 4 and 8 weeks, respectively. The erectile function of animals in the 8-week treatment groups were significantly improved and were comparable with the sham-operated animals, while the results from the 4-week treatment groups were significantly different from the sham values (P < 0.05). Udenafil was effective in preserving erectile function in a time- and dose-dependent manner.

**Gene Expression Profiles**

The mRNA expression of HIF-1α, TGF-β1, NGF, ETB, eNOS, nNOS, and SHH was analyzed by RT-PCR (Figure 2). The level of HIF-1α, TGF-β1, NGF, and ETB mRNA expression was significantly increased by CNCI (P < 0.05). This increase in mRNA expression was markedly suppressed by 4- and 8-week treatment of both doses of udenafil (5 and 20 mg/kg) (P < 0.05). In contrast, CNCI markedly lowered the mRNA expression of eNOS, nNOS, and SHH. Udenafil treatment significantly ameliorated these alterations but the eNOS mRNA expression results from the 4-week treatment group of 5 mg/kg udenafil failed to reach statistical significance.

**Western Blot Analysis**

Protein levels of eNOS, nNOS, and SHH were measured in penile tissue samples. There was a significant decrease in densitometric values of eNOS, nNOS, and SHH in vehicle-treated CNCI group compared with sham-operated animals (P < 0.05). As depicted in Figure 3, 8-week administration of udenafil (5 and 20 mg/kg) significantly ameliorated this decrease (P < 0.05). After a 4-week treatment of udenafil (5 and 20 mg/kg), the protein level of SHH was significantly restored to the sham values, whereas only the high-dose (20 mg/kg) significantly attenuated the decrease in the level of eNOS and nNOS (P < 0.05).

**Immunohistologic Analysis**

Using immunohistochemistry, tissue sections were analyzed for NGF, eNOS, nNOS, CD31, and α-SMA (Figure 4). In line with the results from Western blot analysis, the area of staining for eNOS and nNOS was significantly reduced in the CNCI group compared with the sham group (P < 0.05). However, udenafil treatment preserved the expression of eNOS and nNOS in comparison with the CNCI group (P < 0.05). Both doses of udenafil (5 and 20 mg/kg) significantly attenuated the decrease in CD31 and α-SMA expression caused by CNCI (P < 0.05). The analysis of NGF expression showed significantly increased staining in the animals that underwent CNCI alone when compared with the sham group (P < 0.05). For both of the treatment groups, the staining levels were lower compared with the CNCI group, which was significant for the 20 mg/kg udenafil-treated group (P < 0.05) but not for the 5 mg/kg udenafil-treated group.

**Smooth Muscle/Collagen Ratio**

Smooth muscle/collagen ratio was determined by staining the tissue sections with Masson’s trichrome. CNCI significantly reduced the smooth muscle/collagen ratio compared with the sham-operated animals (P < 0.05) (Figure 5a). Four- and 8-week udenafil treatment was able to significantly restore the smooth muscle/collagen ratio (P < 0.05).

**Apoptosis Analysis**

CNCI induced a marked increase in the apoptotic index. Four-week treatment of 5 and 20 mg/kg udenafil significantly reduced apoptosis to 74.4% and 38.3% of the CNCI values, respectively (P < 0.05). Eight-week treatment suppressed apoptosis to 42.0% and 24.2% of the CNCI group by 5 and 20 mg/kg udenafil, respectively (P < 0.05) (Figure 5b).

**Discussion**

The introduction of nerve-sparing radical prostatectomy has revolutionized the surgical management of organ-confined prostate cancer, and approximately 50,000 cases are performed in the United States each year [5]. However, postoperative erectile dysfunction is yet a major concern for sexually active prostate cancer survivors [17]. The nerve injury that occurs during surgery results in putative endothelial damage [3], and initiates the impairment of the communication between the CN and the penile tissue through wallerian degeneration of the distal axon [18]. Compromised function of the CNs after radical prostatectomy is a dominant cause for atrophy of the corpus cavernosum, loss of neurotransmitters, and fibrosis, which results in erectile dysfunction [3,19]. The rat model of CN injury was first introduced in 1989, and now there are several different models of controlled CN injury, including crushing, freezing, and transection with or without the removal of a segment of the nerve [20]. Advantages of using the crush injury model to evaluate the functional
Figure 2 Gene expression profiles. Reverse transcription-polymerase chain reaction (RT-PCR) amplification for hypoxia-inducible factor (HIF)-1α, transforming growth factor (TGF)-β1, nerve growth factor (NGF), endothelin B receptor (ETβ), endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and sonic hedgehog (SHH) mRNA was performed after 4- or 8-week treatment of udenafil. Cavernous nerve crush injury (CNCI) increased the expression of HIF-1α, TGF-β1, NGF, and ETβ, however, decreased the expression of eNOS, nNOS, and SHH. Chronic administration of udenafil (CNCI + 5 U, 5 mg/kg udenafil treatment group; CNCI + 20 U, 20 mg/kg udenafil treatment group) significantly suppressed these alterations. The densitometry data were normalized by β-actin and expressed as a ratio of sham values. The results are expressed as mean ± standard error. *Significantly different from Sham group (P < 0.05). +Significantly different from CNCI group (P < 0.05).

Figure 3 Western blot analysis of penile tissue extracts. Immunoblots show 140 kDa endothelial nitric oxide synthase (eNOS), 155 kDa neuronal nitric oxide synthase (nNOS), 19 kDa sonic hedgehog (SHH), and 42 kDa β-actin. A significant difference was measured between animals in the cavernous nerve crush injury (CNCI) group and udenafil-treated animals (CNCI + 5 U, 5 mg/kg udenafil treatment group; CNCI + 20 U, 20 mg/kg udenafil treatment group). Data were normalized by β-actin and expressed as a ratio of sham values. The results are expressed as mean ± standard error. *Significantly different from Sham group (P < 0.05). +Significantly different from CNCI group (P < 0.05).
Figure 4  Immunohistochemistry. Representative slides of immunostaining of (a) nerve growth factor (NGF) (b) endothelial nitric oxide synthase (eNOS) (c) neuronal nitric oxide synthase (nNOS) (d) CD31, and (e) α-smooth muscle actin (SMA). The images are at ×200 magnification except for NGF and nNOS (×400 magnification). The results are expressed as the mean ± standard deviation. *Significantly different from Sham group (P < 0.05). †Significantly different from cavernous nerve crush injury (CNCI) group (P < 0.05). CNCI + 5 U, 5 mg/kg udenafil treatment group; CNCI + 20 U, 20 mg/kg udenafil treatment group.
Udenafil and Cavernous Nerve Crush Injury

The high expression of TGF-β1 observed in this study may well be the result of penile flaccidity after CNCI, which a recent report explained as an ongoing neuronal regenerative process with high activation of NGF after CN injury [5]. However, the treatment of the high-dose udenafil attenuated this increase, and further studies are required to elucidate the precise role of this alteration.

CNCI significantly increased the mRNA expression of HIF-1α, TGF-β1, NGF, and ETβ1. HIF-1α activates the transcription of genes encoding proteins crucial for controlling oxygen homeostasis and hypoxia-induced TGF-β1 is reported to increase collagen synthesis in human corpus cavernosal smooth muscle cells [25,29]. TGF-β1 also inhibits smooth muscle cell proliferation via the extension of G2 phase or arrest in the late G2 phase of the cell cycle [30]. ETβ1-mediated vasconstriction has been previously reported and overexpression of ETβ1 mRNA has been observed in several nerve damage studies [31,32]. In this study, chronic treatment of udenafil was shown to significantly suppress these increases. Udenafil treatment was also shown to be beneficial in preserving the mRNA and protein expression of eNOS and nNOS, which is in line with the results from previous studies [33]. Parasympathetic fibers of the CN, which originate in the major pelvic ganglion and proceed in the penile dorsal nerve to the corpus cavernosum, are characterized by their nNOS content. And the loss of nNOS after CNCI that we have observed in this study is in agreement with literature [34].

The effect of PDE5 inhibition on the expression of SHH mRNA and protein has been determined in this study for the first time. In this study, CNCI markedly lowered the level of SHH mRNA and protein expression, and PDE5 inhibition by udenafil administration significantly attenuated this decrease. The role of SHH in penile

recovery of nerves following injury include reliability and reproducibility, and the rat model of CNCI is widely accepted as the most clinically relevant animal model [21].

In this study, CNCI markedly impaired erectile function as compared with the sham animals. Following CNCI, the ICP/MAP ratio was significantly decreased and this was in line with the fibrosis results determined by Masson’s trichrome staining. The Masson’s trichrome staining showed a marked decrease in smooth muscle/collagen ratio. And, as previous studies have reported [5], CNCI significantly increased apoptosis. It is also hypothesized that the absence of early postoperative erections leads to poor cavernosal oxygenation and prolonged hypoxia-induced damage, which promotes corporal fibrosis and result in permanent erectile dysfunction [22]. The decreased penile blood flow may also lead to the apoptosis of cavernosal smooth muscle and endothelial cells, and loss of penile distensibility [23]. Healthy men routinely have nocturnal penile erections. The penile blood partial pressure of oxygen reaches 90–100 mm Hg during erection, whereas it is only 25–40 mm Hg during flaccidity [24]. Nocturnal penile erection retains a high level of penile blood partial oxygen pressure, which may suppress the synthesis of collagen induced by TGF-β1 [25].

The high expression of TGF-β1 observed in this study may well be the result of penile flaccidity after CNCI, and fibrosis observed in animals exposed to CNCI is associated with the increased expression of TGF-β1. This underlines the potential importance of the early initiation of PDE5 inhibitor therapy in patients undergoing radical prostatectomy.

Previous attempts to elucidate the therapeutic role of PDE5 inhibition in CN injury were made with sildenafil [5,26]. Four-week administration of 20 mg/kg sildenafil, but not 10 mg/kg sildenafil, preserved erectile function with statistical significance after CNCI [5]. The authors have also reported a significant reduction in apoptosis by sildenafil treatment at as early as three days after the induction of CN injury. In this study, we have confirmed the restoration of erectile function by udenafil (5 and 20 mg/kg) treatment, and udenafil was also effective in ameliorating fibrosis and apoptosis induced by CNCI. The protective effect of PDE5 inhibitors on CN injury may be a class effect, however, as according to previous studies, not all PDE5 inhibitors are necessarily identical in their cellular and tissue effects and one should be cautious in comparing the effects of PDE5 inhibitors on the preservation of cavernosal tissue integrity without direct comparison experiments [27].

Results from the immunohistologic analysis indicate that the udenafil-induced recovery of erectile function in animals exposed to CNCI is partly mediated by neural and endothelial factors. The staining levels of CD31 and eNOS were markedly decreased by CNCI compared with sham-operated animals, and the daily treatment of 5 mg/kg udenafil was sufficient to significantly restore these levels. These results suggest an endothelial protective effect of udenafil, which is in line with previous reports [28]. In contrast, for NGF, the staining level was significantly increased after CNCI, which a recent report explained as an ongoing neuronal regenerative process with higher activation of NGF after CN injury [5]. However, the treatment of the high-dose udenafil attenuated this increase, and further studies are required to elucidate the precise role of this alteration.
morphogenesis, CN injury-induced apoptosis, and in maintaining CN integrity have been extensively studied by the Podlasek group [35]. Inhibition of SHH function in the penis results in dramatic increase in smooth muscle apoptosis and leads to erectile dysfunction [36,37]. In this study, we observed an increase in SHH expression after the long-term treatment with udenafil in a rat model of CNCI, supporting the regulatory role of SHH on erectile function. However, the decrease in the mRNA expression of SHH after CNCI, which we have confirmed in this study, contradicts the results from certain previous reports [36]. Further studies on the relevant mechanisms are needed to elucidate the effects of this discrepancy, however, the age of animals could have had an effect on the mRNA expression results, as the above-mentioned studies were conducted with relatively older animals. The SHH mRNA level in Sprague Dawley rats are reported to reach its peak at 90 days after birth, and the SHH mRNA level of animals used in this study is approximately 50% of the peak level [37].

Conclusion

Our study shows that the pharmacological effect of udenafil on erectile function recovery is time- and dose-dependent, and offers mechanistic insight into the beneficial effects of udenafil in preserving erectile function after CNCI. Furthermore, this work has clinical implications in the treatment with PDE5 inhibitors in men undergoing radical prostatectomy.

Acknowledgment

This study was supported in part by a Grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ2-PG4-PT01-0024).

Corresponding Author: Kyung-Koo Kang, PhD, Research Laboratory, Dong-A Pharmaceutical Company, 47-5 Sanggal, Giheung, Y ongin, Gyeonggi 446-905, Korea. Tel: 82-31-280-1385; Fax: 82-31-282-8564; E-mail: kangkk donga.co.kr

Conflict of Interest: Chan-Ho Lee, Hae-Sun Kim, Moon-Jung Goo, Kyung-Koo Kang, Byoung-Ok Ahn, and Soon Hoe Kim are currently employees of Dong-A Pharmaceutical Company, Korea.

Statement of Authorship

Category 1

(a) Conception and Design
Chan-Ho Lee; Kyung-Koo Kang; Dae-Yul Yang

(b) Acquisition of Data
Chan-Ho Lee; Hae-Sun Kim; Moon-Jung Goo

(c) Analysis and Interpretation of Data
Chan-Ho Lee; Hae-Sun Kim; Moon-Jung Goo

Category 2

(a) Drafting the Article
Chan-Ho Lee

(b) Revising It for Intellectual Content
Chan-Ho Lee; Kyung-Koo Kang; Byoung-Ok Ahn; Dae-Yul Yang

Category 3

(a) Final Approval of the Completed Article
Byoung-Ok Ahn; Soon Hoe Kim; Dae-Yul Yang

References


1340


