

# Udenafil Enhances the Recovery of Erectile Function and Ameliorates the Pathophysiological Consequences of Cavernous Nerve Resection

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## ABSTRACT

**Introduction.** Radical prostatectomy is the treatment of choice for prostate cancer patients. Despite the introduction of nerve-sparing surgical techniques, its success is not entirely guaranteed and the majority of patients report compromised erectile function following surgical procedures.

**Aim.** This study was performed to investigate the effect of repeated dosing of udenafil, a novel phosphodiesterase type 5 inhibitor, on penile hypoxia and fibrosis induced by bilateral cavernous nerve resection (BCNR) in rats.

**Methods.** Thirty male Sprague-Dawley rats (300–320 g) were used in this study. The animals were divided into three groups; group I consisted of sham-operated animals (N = 10), animals in group II underwent BCNR alone (N = 10), and animals in group III were orally treated with 10 mg/kg udenafil *b.i.d.* for 8 weeks following BCNR (N = 10).

**Main Outcome Measures.** The expression of transforming growth factor- $\beta$ 1, hypoxia-inducible factor-1 $\alpha$ , endothelial nitric oxide synthase, neuronal nitric oxide synthase, and endothelin B receptor in penile tissue was examined at gene level. Additionally, erectile function, measured by intracavernous pressure (ICP), and pathological changes in the corpus cavernosum were examined.

**Results.** While fibrosis, apoptosis, and the expression of TGF- $\beta$ 1, HIF-1 $\alpha$ , and ET<sub>B</sub> were significantly increased, and the expression of eNOS and nNOS were significantly decreased in group II, compared with the sham-operated animals, repeated dosing of udenafil significantly ameliorated these changes. Erectile function was profoundly impaired in animals that underwent BCNR alone, and udenafil treatment significantly attenuated this impairment as measured by ICP.

**Conclusions.** These results demonstrate that long-term administration of udenafil ameliorates penile hypoxia and fibrosis induced by cavernous nerve resection. This study also suggests the potential beneficial role of repeated dosing of udenafil in the recovery of erectile function in patients with neuronal erectile dysfunction. **Lee C-H, Shin J-H, Ahn G-J, Kang K-K, Ahn B-O, and Yoo M. Udenafil enhances the recovery of erectile function and ameliorates the pathophysiological consequences of cavernous nerve resection. J Sex Med 2010;7: 2564–2571.**

**Key Words.** Erectile Dysfunction; Cavernous Nerve Resection; Udenafil; Apoptosis; Fibrosis.

## Introduction

A significant proportion of patients diagnosed with prostate cancer undergo radical prostatectomy for definitive local therapy [1]. Despite advances in surgical techniques, the majority of men report some degree of compromised erectile function following prostatectomy, and the primary pathophysiological mechanism is the

combination of cavernous nerve injury by surgical manipulation and damage to the erectile tissue secondary to neuropraxia or the potential absence of cavernosal oxygenation [2,3]. The nerve injury that occurs during surgery results in putative endothelial damage to the sinusoids and apoptotic loss of corporal smooth muscle cells with a deposition and disorganization of collagen fibers [4–6].

In animal models of cavernous nerve injury, pathophysiological alterations such as reduced nitric oxide synthase (NOS) expression, upregulation of fibroproliferative cytokines, and altered signaling responses have been reported [7–9]. Recent reports have suggested a therapeutic role of phosphodiesterase type 5 (PDE5) inhibition in erectile dysfunction in men who have undergone radical prostatectomy [10,11]. However, the precise pharmacologic mechanism of long-term administration of PDE5 inhibitors is still unclear.

Udenafil (Zydena®, Dong-A Pharmaceutical, Seoul, Korea) is a novel selective PDE5 inhibitor developed as an oral agent for the treatment of erectile dysfunction. With human  $t_{1/2}$  of 11–13 hours and  $t_{max}$  of 1.0–1.5 hours, udenafil shows unique clinical properties of both a rapid onset and a long duration, and a comparable safety profile to other PDE5 inhibitors currently available for treating erectile dysfunction [12,13]. The efficacy and safety of udenafil in men with erectile dysfunction have been documented in previous clinical studies [13,14]. In preclinical studies, udenafil was shown to enhance penile responses in normal, diabetic, and spinal cord injured rabbits via the selective inhibition of PDE5 [15–17], and to ameliorate endothelial dysfunction-related vascular injury [18].

This study was conducted to generate animal data in support of human studies and to define the mechanism of action by which long-term treatment of udenafil ameliorates the functional and histologic alterations induced by bilateral cavernous nerve resection (BCNR) in rats.

## Methods

### Animals and Treatment

Eight-week old Sprague-Dawley rats (300–320 g), obtained from Charles River Laboratories Inc. (Yokohama, Japan), were randomly divided into three groups: (i) sham-operated (Sham, N = 10); (ii) bilateral cavernous nerve resection (BCNR, N = 10); and (iii) 10 mg/kg udenafil treatment group (BCNR + Udenafil, N = 10). Following BCNR, animals in the udenafil treatment group received oral administration of udenafil (10 mg/kg) twice daily, for 8 weeks, by gavage. Other animals were given vehicle only. Udenafil (CAS No.; 268203-93-6, 5-[2-propoxy-5-(1-methyl-2-pyrrolidinylethylaminosulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo(4,3-d)pyrimidine-7-one) was dissolved in Titrisol buffer solution (citrate sodium hydroxide buffer, pH 5.0,

Merck, Darmstadt, Germany). Animals were kept under standard laboratory conditions, and food and ultraviolet-sterilized tap water were provided *ad libitum*. All animal experiments were performed in accordance with the institutional “Standard operating procedure for animal care and experiments” of Dong-A Pharmaceutical Company, as well as with the “Principles of laboratory animal care” established by the National Institutes of Health.

### Surgical Procedures for Bilateral Cavernous Nerve Resection

BCNR was performed as described previously [5]. Animals were anesthetized by intraperitoneal ketamine (100 mg/kg) and xylazine (10 mg/kg) and kept isothermic using a heating pad. After the animals were shaved with surgical clippers, an abdominal incision was made to expose the cavernous nerves and the pelvic ganglia on either side of the prostate. In the sham-operated group, both cavernous nerves were identified but not resected. In the BCNR and BCNR + Udenafil group, the cavernous nerves were isolated and were resected by removing a 5-mm segment. The abdominal incision was then closed layer by layer.

### Evaluation of Erectile Function

The erectile function was assessed by measuring the intracavernous pressure (ICP) by a modification of the method reported by Martinez-Pineiro et al. [19]. After 8 weeks of administration, animals were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and the right carotid artery was exposed and cannulated with a PE-50 tube filled with 50 IU heparinized saline to monitor the mean arterial pressure (MAP). The penile crura were exposed through a perineal–scrotal and prepuce incision by spreading the overlying ischio-cavernous muscle. A 26G needle connected to polyethylene tubing filled with heparinized saline was inserted into the corpus cavernosum to measure the ICP. Erection was achieved by a direct injection of 10  $\mu$ mole and 100  $\mu$ mole sodium nitroprusside (SNP) into the corpus cavernosum. MAP and ICP were recorded on a polygraph and the data acquisition and calculation of the derived parameters were performed using a signal processor and were presented as the maximum ICP/MAP (%). This measurement was carried out 24 hours after the last administration to eliminate the acute effects of udenafil, which has a  $t_{1/2}$  of 3–4 hours in rats.

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

Gene	Forward primer (5'→3')	Reverse primer (5'→3')	Product size (bp)
TGF-β1	CCTGGAAGGGCTCAACACC	GTTGGACAACCTGCTCCACCT	352
HIF-1α	GTCTCGAGATGCAGCCAGATCTCG	GGTCAGATGATCAGAGTCCAAAGC	800
eNOS	CAGGCTGCCTGTGAAACTTT	CATCCTGGGTTCTGTATGCC	790
nNOS	CCTTCCGAAGCTTCTGGCAACAGC	TGGACTCAGATCTAAGGCGGTTGG	473
ET <sub>B</sub>	TTCACCTCAGCAGGATTCTG	AGGTGTGGAAAGTTAGAACG	474
β-Actin	TCTACAATGAGCTGCGTGTG	AGGATGGCATGAGGGAGC	259

bp = base pair; TGF-β1 = transforming growth factor-beta 1; HIF-1α = hypoxia-inducible factor-1 alpha; eNOS = endothelial nitric oxide synthase; nNOS = neuronal nitric oxide synthase; ET<sub>B</sub> = endothelin B receptor.

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

Freshly dissected samples of corpus cavernosum from 8-week-treated animals were subjected to RT-PCR amplification for transforming growth factor (TGF)-β1, hypoxia-inducible factor (HIF)-1α, endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and endothelin B receptor (ET<sub>B</sub>) mRNA. Total RNA samples were isolated using Trizol (Invitrogen, Carlsbad, CA, USA) and quantified by the following steps. The primer sequences used for PCR amplification are shown in Table 1.

Isolated RNA samples were reverse-transcribed to first strand cDNA at 48°C for 45 min, and RNA/cDNA hybrids were denatured by 2-min incubation at 94°C. The amplification cycling conditions were as follows: consisted of 35 cycles of denaturation (94°C, 30 seconds), template/primer annealing (eNOS: 54°C, nNOS: 52°C for 1 min), and extension (68°C, 2 minutes). All procedures were carried out using the Access RT-PCR system (Promega, Madison, USA). To verify the PCR products, 1% agarose gel electrophoresis and ethidium bromide staining were applied. Densitometric analysis was carried out using the BIO-PROFIL system and BIO-1D image analysis software (Wilber Lourmat, Torcy, France).

### Histologic Analysis

Corporal tissues from each group were fixed in 10% buffered formalin for morphologic analysis. Sections embedded in paraffin were stained by Masson's trichrome staining for smooth muscle/collagen ratio as described previously [20]. Terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) assay was used to detect apoptosis. Deparaffinized tissue sections were analyzed using an *In situ* Apoptosis Detection Kit (Takara, Shiga, Japan) according to the manufacturer's instructions. Two tissue sec-

tions from 5 different animals per group were randomly selected and reviewed. Therefore, in each group, a total of 10 sections were analyzed.

### Statistical Analysis

The overall significance of the experimental results was analyzed by analysis of variance (ANOVA) test. The results are presented as mean ± standard deviation and comparisons between the group mean values were carried out using Kruskal-Wallis ANOVA on ranks at a statistical significance level of  $P < 0.05$ .

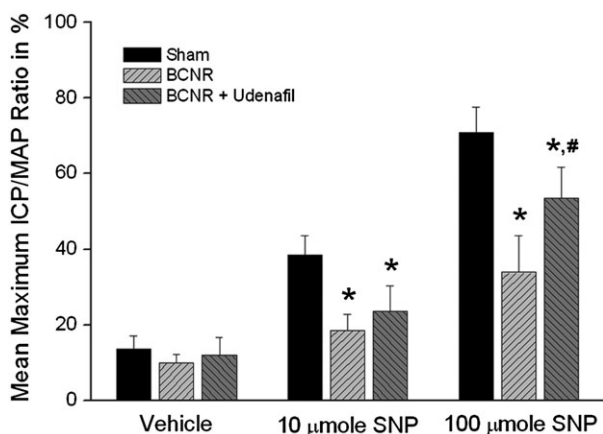
## Results

### Erectile Function

In order to evaluate the changes in erectile function, ICP, which correlates with penile rigidity, was measured. The intracavernosal injection of SNP, which induced erection in rats, had little effect on the systemic blood pressure and no significant changes were observed in the MAP data. The results were expressed as the mean maximum ICP/MAP ratio. As shown in Figure 1, BCNR induced a significant decrease in ICP ( $P < 0.05$ ). When SNP was directly injected into the corpus cavernosum at 100 μmole, the ICP/MAP ratio in sham-operated animals and in BCNR animals were  $70.8 \pm 6.9\%$  and  $34.0 \pm 9.8\%$ , respectively. Eight-week administration of 10 mg/kg udenafil significantly ameliorated this decrease, and the ICP/MAP ratio of the BCNR + Udenafil group was  $53.4 \pm 8.3\%$  ( $P < 0.05$ ).

### TGF-β1 and HIF-1α mRNA expression

Total RNA samples extracted from the penile tissues of rats were analyzed by RT-PCR to investigate the mRNA expression of TGF-β1 and HIF-1α. Figure 2 shows the mRNA levels of TGF-β1 and HIF-1α. The level of TGF-β1 and HIF-1α mRNA expression was markedly increased by 2.65- and 1.99-fold, respectively, after BCNR



**Figure 1** Erectile function results after 8-week udenafil treatment in animals exposed to bilateral cavernous nerve resection (BCNR). Erection was achieved by a direct injection of sodium nitroprusside (SNP) into the corpus cavernosum. The results are shown as the mean maximum intracavernous pressure (ICP)/mean arterial pressure (MAP) ratio as a percentage for each group. The results are expressed as mean ± standard deviation. \*Significantly different from Sham group ( $P < 0.05$ ). #Significantly different from BCNR group ( $P < 0.05$ ).

( $P < 0.05$ ). This increase in mRNA expression was significantly suppressed by udenafil treatment ( $P < 0.05$ ).

**eNOS, nNOS, and ET<sub>B</sub> mRNA Expression**

In the BCNR group, the ratios of band density of eNOS and nNOS mRNA to β-actin were signifi-

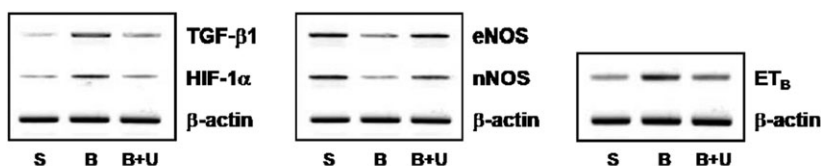
cantly decreased compared with the sham-operated group ( $P < 0.05$ ). In comparison with the BCNR group, 8-week treatment of 10 mg/kg udenafil significantly increased the mRNA levels of eNOS and nNOS in the penile tissues ( $P < 0.05$ ). On the contrary, the ET<sub>B</sub> mRNA was overexpressed after BCNR by 1.98-fold compared with sham ( $P < 0.05$ ), and this increase was significantly suppressed by udenafil ( $P < 0.05$ ) (Figure 2).

**Smooth Muscle/Collagen Ratio**

Smooth muscle/collagen ratio was determined by staining the tissue slides, showing the cross-section of the corpora cavernosa, with Masson’s trichrome. As depicted in Figure 3, BCNR significantly reduced the smooth muscle/collagen ratio by 37.5%, compared with the sham-operated animals ( $P < 0.05$ ). Long-term udenafil treatment was able to markedly augment the smooth muscle/collagen ratio to 87.5% of the sham group ( $P < 0.05$ ).

**Apoptosis Analysis**

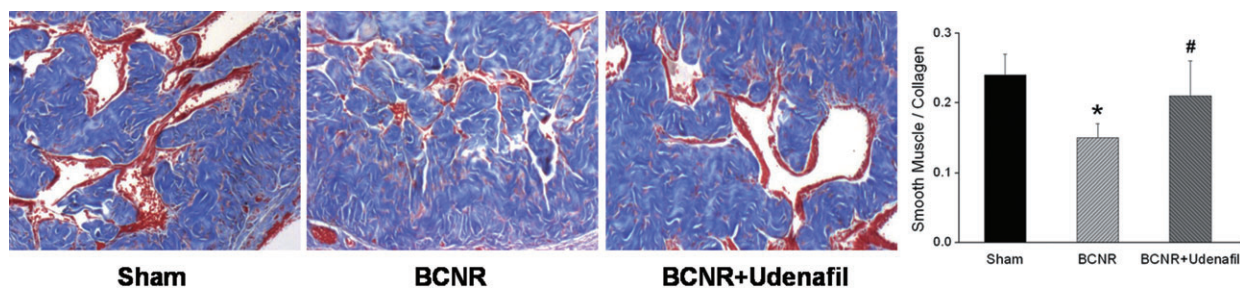
After 8 weeks from the day BCNR was performed, the ratio of apoptotic/total cells increased to  $0.27 \pm 0.06$ , which is significantly higher compared with  $0.07 \pm 0.02$  in the sham-operated group ( $P < 0.05$ ). Eight-week administration of udenafil significantly reduced apoptosis induced by BCNR. The apoptotic/total cells ratio for the



**Quantified blot densities (arbitrary unit)**

Genes	Sham (S)	BCNR (B)	BCNR+Udenafil (B+U)
TGF-β1	1.00±0.18	2.65±0.20*	1.58±0.20#
HIF-1α	1.00±0.16	1.99±0.11*	1.25±0.10#
eNOS	1.00±0.10	0.59±0.20*	0.83±0.10#
nNOS	1.00±0.09	0.48±0.09*	0.76±0.05#
ET <sub>B</sub>	1.00±0.14	1.98±0.30*	1.54±0.11#

**Figure 2** Gene expression profiles. Reverse transcription-polymerase chain reaction (RT-PCR) amplification for transforming growth factor (TGF)-β1, hypoxia-inducible factor (HIF)-1α, endothelial nitric oxide synthase (eNOS), neuronal nitric oxide synthase (nNOS), and endothelin B receptor (ET<sub>B</sub>) mRNA was performed after an 8-week treatment of udenafil. The level of TGF-β1, HIF-1α, and ET<sub>B</sub> mRNA expressions was markedly increased, and mRNA expressions of eNOS and nNOS were markedly decreased by bilateral cavernous nerve resection (BCNR). Eight-week treatment of 10 mg/kg udenafil significantly reversed these alterations. The results are expressed as mean ± standard deviation. \*Significantly different from Sham group ( $P < 0.05$ ). #Significantly different from BCNR group ( $P < 0.05$ ).



**Figure 3** Masson's trichrome staining. Fibrosis was determined by staining tissue samples with Masson's trichrome. The results are shown as smooth muscle/collagen ratio. The images depict representative fields and the bar chart shows the results from quantitative image analysis. The results are expressed as mean  $\pm$  standard deviation. \*Significantly different from Sham group ( $P < 0.05$ ). #Significantly different from bilateral cavernous nerve resection (BCNR) group ( $P < 0.05$ ).

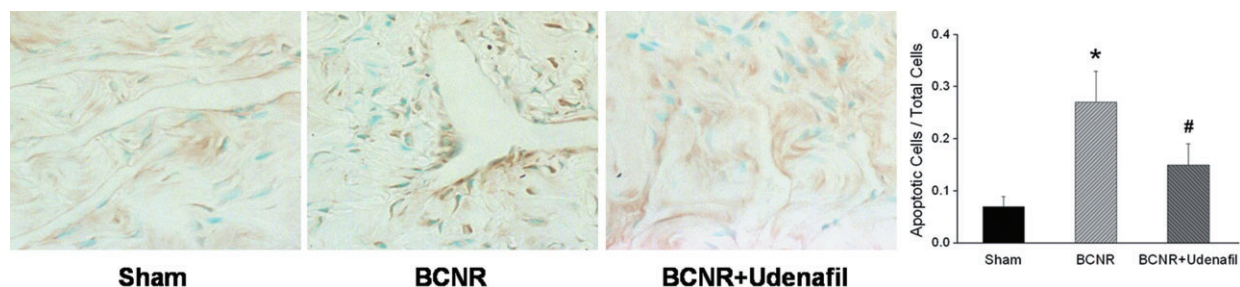
BCNR + Udenafil group was  $0.15 \pm 0.04$  ( $P < 0.05$ ) (Figure 4).

### Discussion

Despite the advent of nerve-sparing radical prostatectomy, many men yet suffer from this operative complication—erectile dysfunction [21]. Although the high rate of erectile impairment following prostatectomy is well acknowledged, the pathophysiology and etiology are yet to be elucidated. The common consensus is that the nerve damage caused by radical prostatectomy initiates the impairment of the communication between the cavernous nerve and the penile tissue through wallerian degeneration of the distal axon [22]. Previous studies have shown a marked improvement in erectile function in rat models of cavernous nerve transection, where reconstruction was performed with grafted genitofemoral nerves [23,24]. Compromised cavernous nerve function after radical pelvic surgery for prostate, invasive bladder or colorectal malignancies is a major cause for atrophy of

the corpus cavernosum, fibrosis, collagen accumulation, and a loss of key neurotransmitters, which leads to erectile dysfunction [5,25,26].

Following penile denervation in rats, apoptotic cell death occurred in the erectile tissue of the penis as early as 2 days following denervation [6], and similar histologic changes were observed in the human penis after radical prostatectomy [4]. Apoptotic cell death may be a crucial event leading to the lack of recovery or response to pharmacologic therapies [27]. Moreover, inflammatory response follows, suggesting endogenous tissue repair, and reactive oxygen species are generated in response to this inflammation, which aggravates penile tissue damage [28]. Pathophysiologic mechanisms include lack of nitric oxide release from cavernous nerve projections to the penis, compromised synthesis of presynaptic nitric oxide, changes in target tissue, or a combination of these factors [29]. Among the various animal models, it is recognized that nerve excision method is a steadfast method as animals exposed to other models tend to exhibit spontaneous erectile function



**Figure 4** Terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling (TUNEL) assay. TUNEL assay was used to detect apoptosis. Bilateral cavernous nerve resection (BCNR) significantly increased apoptosis, and this increase was suppressed by udenafil treatment. The images depict representative fields and the bar chart shows the results from quantitative image analysis. The results are expressed as mean  $\pm$  standard deviation. \*Significantly different from Sham group ( $P < 0.05$ ). #Significantly different from BCNR group ( $P < 0.05$ ).

recovery even without treatment [30]. BCNR represents the most radical surgical procedure for penile denervation and is considered the representative model to study erectile dysfunction induced by interruption of the autonomic nerve supply to the corpus cavernosum [31,32].

In this study, BCNR elicited a dramatic impairment of the erectile function compared with the sham-operated animals. The reduction in the ICP/MAP ratio was in line with the results from Masson's trichrome staining for the evaluation of fibrosis. The smooth muscle/collagen ratio, indicative of collagen accumulation, fibrosis, was significantly decreased in the BCNR group. An increase in apoptosis was also observed in BCNR animals and these observations are consistent with previous reports [5,33]. It is theorized that apoptosis induced by cavernous nerve injury involves the smooth muscle cells, and as a result, leads to occlusive alterations in the vein and erectile dysfunction [5].

Previous attempts to elucidate the therapeutic role of PDE5 inhibition in a rat model of BCNR were made with tadalafil and sildenafil [34,35]. Three-month administration of tadalafil amplified nitric oxide signaling, which helped to preserve PDE5 expression and thus rescued sensitivity to acute tadalafil [34], and exposure to sildenafil was able to counteract the alterations observed after BCNR, with the effect of sildenafil being more evident the earlier it was administered [35]. In this study, we have confirmed the recovery of erectile function by udenafil (10 mg/kg) treatment, and udenafil was also effective in ameliorating fibrosis and apoptosis induced by BCNR. And, as the efficacy of udenafil depended upon nitric oxide provided by SNP, which replaced the naturally released nitric oxide, the efficacy of udenafil is suggested to result during sexual arousal. However, further studies are required to evaluate the precise mechanism of these therapeutic effects of udenafil.

While healthy men routinely have roughly four phases of nocturnal penile erection, hypoxia is induced in the penile tissue of patients with neurogenic erectile dysfunction. During erection, the penile blood partial pressure of oxygen reaches 90–100 mm Hg; whereas, it is only 25–40 mm Hg during flaccidity [36]. Nocturnal penile erection is able to maintain a high level of penile blood partial oxygen pressure, which may suppress the synthesis of collagen induced by TGF- $\beta$ 1 [37]. The high expression of TGF- $\beta$ 1 observed in this study may well be the result of penile flaccidity after BCNR, and the fibrotic change observed in animals

exposed to BCNR is closely related to the increased expression of TGF- $\beta$ 1. TGF- $\beta$ 1 has various roles in inflammation, stimulation of intercellular matrix formation, production of fibroblast, and healing [38]. TGF- $\beta$ 1 is upregulated in ischemic conditions and there are reports of increased collagen synthesis in human corpus cavernosal smooth muscle cells by hypoxia-induced TGF- $\beta$ 1 [37,39]. HIF-1 $\alpha$  is a transcription factor expressed in mammalian cells under hypoxic conditions, and activates the transcription of gene-encoding proteins that are crucial for maintaining oxygen homeostasis [40]. The results of this study show markedly increased expression of TGF- $\beta$ 1 and HIF-1 $\alpha$  mRNA in the BCNR group. The compromised erectile function resulted in an insufficient oxygen supply to the penile tissue, and in this study, udenafil was shown to be beneficial, markedly suppressing the increase in TGF- $\beta$ 1 and HIF-1 $\alpha$  mRNA expressions.

Nitric oxide, a major factor in the initiation of penile erection by inducing smooth muscle relaxation in the corpus cavernosum, is tightly regulated by nNOS in penile neurons innervating the corpus cavernosum [41]. eNOS is active in penile vascular endothelial cells and in trabecular meshwork activated by blood flow-mediated shear stress [42]. Previous studies performed in animal models of cavernous nerve injury have reported decreases in eNOS and nNOS expressions [26]. This study shows that the daily treatment of udenafil preserves the level of eNOS and nNOS mRNA expressions. These results are supported by the previous sildenafil study performed by Musicki et al. in which the authors made a conclusion that PDE5 inhibition enhances Akt-dependent eNOS phosphorylation [43].

Endothelin-1 (ET-1) expression is reported to be upregulated by several factors including TGF- $\beta$ 1, ET-1 itself, and by prolonged hypoxia [44]. ET<sub>B</sub> mRNA and protein overexpression has been reported in several nerve injury studies, which is in line with our results [34]. Although ET<sub>B</sub> are commonly accepted to mediate the dilatatory response to ET-1 via the activation of nitric oxide production, recent reports have shown vasoconstriction mediated by ET<sub>B</sub> [45]. The diverse actions of ET<sub>B</sub> have been explained by the existence of two putative receptor subtypes: ET<sub>B1</sub> and ET<sub>B2</sub>. Whether these subtypes are truly distinct receptor subtypes or the same receptor expressed on different cells and with different downstream signal transduction events is yet to be elucidated.

Interestingly, chronic exposure to other PDE5 inhibitors, sildenafil and tadalafil, have demonstrated similar results on penile oxygenation despite some inconsistencies in clinical trial reports [34,46–48]. Action of udenafil, sildenafil, and tadalafil on PDE5 signifies that some cyclic guanosine monophosphate is yet formed, even in an experimental model characterized by a complete denervation, which significantly decreases eNOS and nNOS expressions [34].

## Conclusion

This study is the first work to demonstrate the therapeutic advantage of long-term administration of udenafil, a novel selective PDE5 inhibitor, in a cavernous nerve resection model. Udenafil clearly enhanced the erectile function by ameliorating penile fibrosis and reducing apoptosis of smooth muscle cells induced by cavernous nerve damage, and these results are in agreement with previous studies performed with other PDE5 inhibitors. However, the present findings should be confirmed in other erectile dysfunction models induced by cavernous nerve injury. The potential role of udenafil as a therapeutic agent in patients with cavernous nerve injury will be elucidated in future clinical trials to assess a possible therapeutic strategy to counteract the erectile dysfunction.

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*Conflict of Interest:* None declared.

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