해면체신경 손상 발기부전 백서 모델에서 JNK 억제제와 HDAC 억제제 병합 치료를 이용한 음경해면체 세포고사, 섬유화 억제와 발기능 호전

Restoring erectile function by combined treatment with JNK inhibitor and HDAC inhibitor in a rat model of cavernous nerve injury

2022년 8월

서울대학교 대학원

의학과 비뇨의학 전공

이 정 훈

해면체신경 손상 발기부전 백서 모델에서 JNK 억제제와 HDAC 억제제 병합 치료를 이용한 음경해면체 세포고사, 섬유화 억제와 발기능 호전

지도 교수 손 환 철

이 논문을 의학박사 학위논문으로 제출함
2022년 4월

서울대학교 대학원
의학과 비뇨의학 전공
이 정 훈

이정훈의 의학박사 학위논문을 인준함
2022년 7월

위 원 장 김 수 웅 (인)

부위원장 손 환 철 (인)

위 원 오 윤 규 (인)

위 원 문 민 환 (인)

위 원 류 지 간 (인)

초 록

해면체신경 손상 발기부전 백서모델에서 JNK 억제제와 HDAC 억제제 병합 치료를 이용한 음경해면체 세포고사, 섬유화 억제와 발기능 호전

이정훈

의학과 비뇨의학 전공

서울대학교 대학원

배경 및 서론

근치적 전립선 적출술 후 발생하는 발기부전의 주요 병태생리는 수술 중 해면체신경의 손상이 음경해면체의 섬유화 및 세포고사를 유도하기 때문이라고 알려져 있다. 이에 해면체신경 손상으로 유발된 발기부전 백서모델에서 JNK억제제 (SP600125)와 HDAC억제제 (suberoylanilide-hydroxamic-7 acid)를 2주간 병합 치료하였을 때 음경해면체의 섬유화 억제 및 세포고사 개선을 통해 발기력을 회복시킬 수 있는지 알아보고자 하였다.

대상 및 방법

12주령의 웅성 Sprague-Dawley 백서 70마리를 5군으로 나누어 2주간 실험하였다. 각 군 (군당 14마리)은 1) Sham 대조군, 2) 양측 해면체신경 손상군, 3) 양측 해면체신경 손상 직후부터 10mg/kg JNK억제제를 일일 1회 복강 내 투여군, 4) 양측 해면체신경 손상 직후부터 25mg/kg HDAC억제제를 일일 1회 경구 투여한 군, 5) 양측 해면체신경

손상 직후부터 10mg/kg JNK억제제 일일 1회 복강 내 투여와 함께 25mg/kg HDAC억제제를 일일 1회 경구 투여한 군으로 구분하였다. 각 군을 2주간 유지한 후 군당 7마리의 백서에게 전기자극에 대한 평균동맥압에 대한 최대해면체내압의 비 (ICP/MAP)와 해면체내압의 곡선의 비 (AUC/MAP)측정으로 발기반응을 평가하였다. 그리고 나머지 7마리에서 적출한 음경조직으로 Masson’s trichrome 염색, 면역화학 염색, Caspase-3 활성 어세이, Western blot 분석을 시행하였다.

결과

양측 해면체신경 손상군은 전기자극 시 ICP/MAP와 AUC/MAP 감소, 평활근/콜라겐 비율 감소, 평활근 함량 감소, Caspase-3 활성 증가, HDAC3 단백발현 증가, TGF-β 단백발현 증가, fibronectin 단백발현 증가, c-Jun 인산화 증가 등이 Sham 대조군에 비해 유의한 소견으로 관찰되었다. 세 치료군 모두에서 신경 손상군에 비해 발기반응, 평활근/콜라겐 비율에서 호전을 보였다. 특히, 병합 치료군은 단독제제 치료군들에 비해 1.0V자극에 대한 발기반응과 평활근/콜라겐 비율에서 추가적인 호전을 보였다. JNK억제제가 투여된 군들은 평활근 함량, Caspase-3 활성, c-Jun 인산화에서 호전을 보였다. HDAC억제제가 투여된 군들은 HDAC3, TGF-β, fibronectin 들의 단백발현이 정상화되었다.

결론

해면체신경 손상 백서모델에서 급성기 동안 JNK억제제와 HDAC억제제 병합 치료는 HDAC/TGF-β 경로와 JNK 경로를 정상화함으로써 해면체의 섬유화와 세포고사를 억제하여 발기능을 회복시킬 수 있었다.

주요어: 세포고사, 섬유화, 발기부전, 히스톤 탈아세틸화효소, Jun 아미노말단 인산화효소, 전립선적출술

학번: 2019-34092

Contents

List of Figures or Tables

Introduction

Prostate cancer and advances in radical prostatectomy

Prostate cancer is the most common cancer in men in 114 countries, including the United States (Global Burden of Disease Cancer et al., 2019; Siegel, Miller, & Jemal, 2020). Eighty to ninety percent of these patients are classified as having clinically localized prostate cancer, a cancer confined to the prostate (T1 or T2), and surgical treatment is one of the standard treatments for clinically localized prostate cancer (Mohler et al., 2019).

After radical prostatectomy (RP) using the perineal approach was introduced in 1905, the current surgical method was established as anatomical knowledge deepened (Lepor, 2005). The advent of the retropubic approach and the introduction of the early ligation technique of the dorsal vein complex discovered through anatomical researches provided a favorable surgical field to the surgeon and significantly less intraoperative bleeding. Since then, RP has been established as the standard treatment for localized prostate cancer with significantly reduced intraoperative and postoperative complications. Currently, it is reported that the cancer-specific mortality rate after RP is as low as 2%, and most patients can return to their daily life (Mohler et al., 2019).

Erectile dysfunction after radical prostatectomy

Erectile dysfunction (ED), which is one of the causes of lower quality of life after surgery, occurs in 20–90% of patients with radical prostatectomy(Philippou et al., 2018). It is known that ED occurs in over 70% of patients with non-nerve-sparing procedure, and in 30–50% of patients even with nerve-sparing procedures during surgery (Dorin et al., 2012; Ficarra et al., 2009; Meyer, Gillatt, Lockyer, & Macdonagh, 2003; Resnick et al., 2013). Erectile dysfunction patients after RP responded that 50% had a 'moderate' decrease and 20% had a 'severe' decrease in quality of life (Meyer et al., 2003). Various psychosocial difficulties such as low self-esteem, blame, guilty, anger, and unhappiness were complained of during the median 92 months after surgery. And 71% of ED patients were disappointed with their partner's response to erectile dysfunction. Singer et al. reported that 70% of prostate cancer patients prefer to reduce the probability of ED by 30% with RT, even if they give up a 10% survival increase with surgery (Singer et al., 1991).

Nerve-sparing prostatectomy and penile rehabilitation

Human fetus and cadaver studies for ED after RP identified cavernosal nerve that courses along the prostate and regulate erection (Lepor, Gregerman, Crosby, Mostofi, & Walsh, 1985; Walsh, Lepor, & Eggleston, 1983). This nerve originates from the

pelvic plexus and innervates the corporal cavernosum along the posterolateral aspect of the prostate capsule. Based on this anatomical knowledge, a nerve-sparing technique was established for the preservation of the neurovascular bundle containing the cavernosal nerve. The penile rehabilitation such as medications, devices and activities were introduced and tried to improve the recovery period and intensity of erectile function after surgery for two decades (Philippou et al., 2018; Sari Motlagh et al., 2021). Although various efforts for penile rehabilitation, including the use of 5 phosphodiesterase inhibitors (PDE5 inhibitors), have been attempted, they are not optimal for use in clinical practice because the effect of current treatment is insufficient (Fode, Ohl, Ralph, & Sonksen, 2013; Wang et al., 2014). Therefore, it is necessary to study mechanism-specific targeted therapies that can specifically inhibit apoptosis and fibrosis.

The pathophysiology of post-RP ED

The pathophysiology of post-RP ED is considered as a combination of several causative factors such as cavernosal nerve (CN) injury, arterial injury and venogenic cause. Among these factors, apoptosis and fibrosis induced by neuropraxia or CN injury caused by pulling and compression during surgery, are considered as a main pathophysiology of structural alteration in the penile corpus tissue (Figure 1). What has been found to date is that

structural alterations in the penile corpus tissue causes venous leakage, known as cavernosal veno-occlusive dysfunction (CVOD). The neuropraxia as an initiating point of CVOD also reduces the bioavailability of neuronal nitric oxide (Capogrosso, Salonia, Briganti, & Montorsi, 2016; Cohen & Glina, 2015; Ferrini et al., 2009; Fode et al., 2013; User, Hairston, Zelner, McKenna, & McVary, 2003). Therefore, the goal of penile rehabilitation for post-RP ED is to prevent structural alterations caused by apoptosis and fibrosis as a result of neuropraxia.

Targeted apoptosis and fibrosis pathways to prevent cavernosal veno-occlusive dysfunction

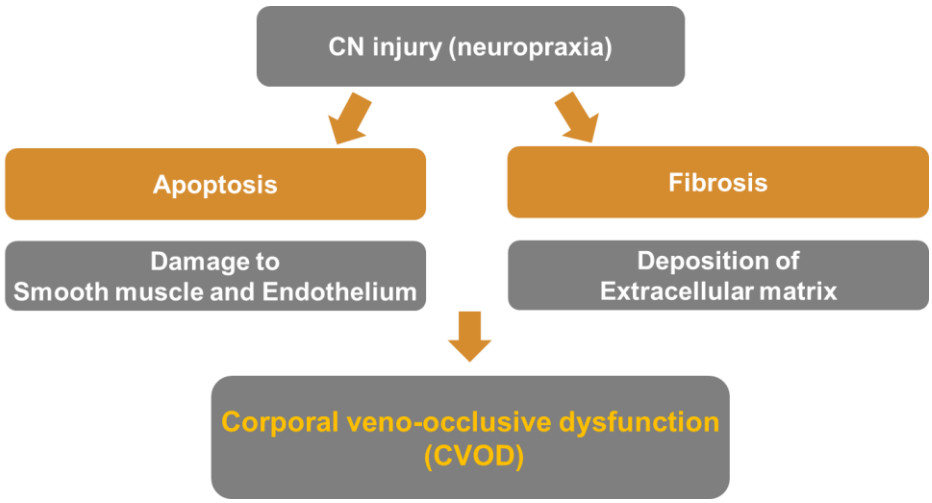
Inhibition of apoptosis and fibrosis has been studied using Rho-kinase inhibitors, angiotensin-II antagonists, TGF-β inhibitor, LIM domain kinase 2 (LIMK2) inhibitor, stem cell, and sonic hedgehog for the treatment of erectile dysfunction in a rat model of CN injury. However, another effective target selection is required due to insufficient efficacy, safety and data of previous substances (Salonia et al., 2017a, 2017b). JNK induces apoptosis by activating c-JUN, Bcl2 and Bax through various pathways in the cell (Dhanasekaran & Reddy, 2008; L. Liu et al., 2012). We reported a partial improvement in erectile function by suppressing apoptosis with Jun-amino terminal kinase inhibitor (JNKi) administration in a rat model of CN crush injury (CNCI) (J. Park, Chai, Kim, Paick, &

Cho, 2018). Fibrosis has been found to be caused by various mechanisms, and histone deacetylase (HDAC) has recently been noted as a closely related molecule (Glenisson, Castronovo, & Waltregny, 2007). HDAC induces fibrogenesis by fibroblast-to-myofibroblast transformation through TGF-β signaling, a key molecular pathway of fibrosis. Our previous studies reported the possibility of mechanism-specific targeted therapies by JNKi for apoptosis or HDAC inhibitor (HDACi), suberoylanilide hydroxamic acid (SAHA; a pan-HDACi), for fibrosis using a CNCI rat model (Cho, Lee, Son, & Kim, 2020; J. Park, Chai, et al., 2018).

Purpose of this study

By focusing on the anti-apoptotic effect of JNKi or the anti-fibrotic effect of the HDACi, we hypothesized that the combined inhibition of JNK and HDAC could improve erectile function by normalizing each signaling pathway. In this study, we investigated whether the combined administration of JNKi and HDACi (SAHA) could restore erectile function by suppressing both apoptosis and fibrosis of the corpus cavernosum.

Figure 1. A schematic diagram demonstrating that the process of tissue alteration caused by neuropraxia during radical prostatectomy induces corporal veno-occlusive dysfunction of corpus cavernosum



Materials and Methods

Animals and study design

A total of 70 12-week-old male Sprague-Dawley rats (weight, 300-350 g) were randomized into five groups : (1) Sham surgery group (n=14): S group, (2) Bilateral CNCIs group (n=14): I group, (3) Bilateral CNCIs treated with JNKi for 2 weeks (n=14): J group, (4) Bilateral CNCIs treated with HDACi for 2 weeks (n=14): H group, (5) Bilateral CNCIs treated with JNKi and HDACi for 2 weeks (n=14): J+H group (Figure 2). The rats were anesthetized with intraperitoneal injections of 10 mg/kg zoletil (Vibac Laboratories, Carros, France) and inhalation of isoflurane, and then the lower abdominal midline was incised. The S group received daily intraperitoneal injection and oral administration of saline vehicle (25% dimethylsulfoxide in saline) after exposing bilateral CNs without any direct injury. The I group received daily intraperitoneal injection and oral administration of saline vehicle after bilateral CNCIs. For CN crush injuries (mechanical compressions), a microsurgical vascular clamp was applied to the CN 4 – 5 mm distal to the bilateral major pelvic ganglions (MPGs) for 80 seconds, removed for 30 seconds, then reapplied for an additional 80 seconds. In the J group, 10 mg/kg of JNKi (SP600125, Selleckchem, USA) was injected intraperitoneally once a day, and

saline vehicle was orally administered (J. Park, Chai, et al., 2018). The H group received oral administration of 25 mg/kg of HDACi (SAHA, Vorinostat, Selleckchem, USA) once daily and daily intraperitoneal injection of saline vehicle (Cho, Lee, Son, et al., 2020). The J+H group was administered a combination of 10 mg/kg JNKi and 25 mg/kg HDACi.

Preparing tissues for evaluation

After 2 weeks of administration, erectile function, histology, and protein expression levels were measured in seven rats in each group. The corpus cavernosum was excised from the other seven animals, and the tissue was examined to evaluate histological changes and the protein expression level of the target molecular pathway related to apoptosis and fibrosis. For histological evaluation, two tissue sections with a thickness of 2.5 mm per animal were harvested from the middle part of skin-denuded penile shaft. Tissue slides were prepared by keeping the penile middle part of the denuded shaft in a 10% formaldehyde solution overnight and then placing them in paraffin wax. The remaining penile tissues were stored at −80° C. Caspase-3 activity assay and western blot analysis were performed using the remaining tissues after the completion of histological evaluation. All procedures were performed by an expert operator using the same method. This experiment was approved by the Institutional Animal Care and Use

Committee of the Clinical Research Institute of our hospital and was conducted in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) certified facility (IACUC number: 17-0147-S1A0).

Erectile function evaluation

Intracavernosal pressure (ICP) and mean arterial pressure (MAP) were assessed by evaluating the erectile response to electrostimulation of the CNs, as described in previous studies (J. Park, Chai, et al., 2018; J. Park, Cho, et al., 2018; K. Park, Ryu, Li, Kim, & Paick, 2008). To continuously monitor the MAP, a 24-gauge angiocatheter was placed in the carotid artery. The ICP was continuously measured by cannulating a 26-gauge needle into the corpus cavernosum. We placed a platinum bipolar electrode into the CN at the distal site from the nerve injury site, which was activated at 1.0, 3.0, and 5.0 V at 12 Hz with 0.2 millisecond duration of square wave for 30-s and a 5 min interval. The erectile responses were evaluated as the maximum ICP/MAP, and the area under the curve (AUC)/MAP ratio was measured during electrostimulation.

Histologic evaluation of fibrosis

To calculate the SM/collagen ratio, slides were stained for Masson's trichrome according to a standard protocol. For Masson's trichrome staining, ×40 magnification images of the

penis comprising one-half of the corpora cavernosa were analyzed for SM (stained in red) and collagen (stained in blue) using Image Pro Plus 4.5 software (Medica Cybernetics, Silver Spring, MD, USA). After quantifying the composition of collagen and SM, the SM/collagen ratio was evaluated.

Histologic evaluation of apoptosis

We evaluated SM content labeled with primary antibody to α-SM actin (α-SMA) (1:100, Dako, Glostrup, Denmark) by immunohistochemical staining. And the percentage of SM fibers was measured. Each image was evaluated by an independent researcher as a blind test. These processes were performed in the same manner as previously described (J. Park, Chai, et al., 2018; J. Park, Cho, et al., 2018). After histologic evaluation, protein levels of molecules related to fibrotic and apoptotic pathways were analyzed by caspase-3 activity assay and western blotting in penile tissues of each group (K. Park et al., 2008).

Caspase-3 activity assay to determine the degree of apoptosis

We determined the activity of caspase-3, a critical executioner of apoptosis, using a caspase-3 activity assay. Caspase-3 activity was quantified using a caspase-3 colorimetric enzyme-linked immunosorbent assay kit (Catalog No. ab39401, Abcam, Cambridge,

UK).

Cavernous tissue samples were homogenized in Cell Lysis Buffer (pH 7.5). Then, a 50 μL aliquot of the supernatant obtained by centrifugation at 10,000 x g for 1 minutes was used to prepare a cell sample according to the method recommended by the manufacturer. The Pierce™ BCA Protein Assay Kit (Catalog No. 23227: Thermo Scientific™, Waltham, USA) was used for accurate determination of protein concentrations, according to the method recommended by the manufacturer. A 50 μL of 2x Reaction Buffer (containing 10mM dithiothreitol) and a 5 μL of the 4 mM DEVD-p-NA substrate were added to each sample. They were mixed and incubated at 37℃ for 90 min. We measured the output at OD 400 nm on a microplate reader. The level of caspase-3 activity in each group is presented as a fold change over the control (S group).

Western blot analysis to evaluate the protein expression level of each target pathway

The protein expression levels of signaling molecules related to apoptosis (JNK pathway: c-Jun) and fibrosis (HDAC/TGF-β pathway: TGF-β, HDAC3 and fibronectin)were evaluated by western blot analysis.

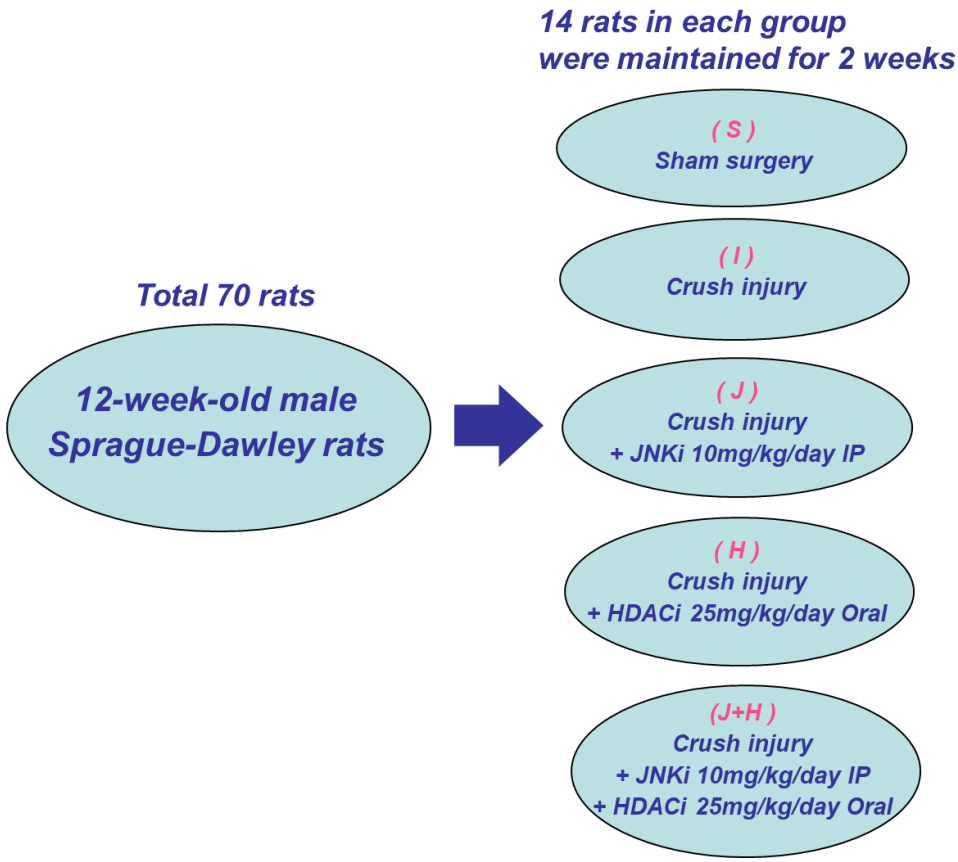
After homogenizing the tissue samples to obtain protein, 25 μg of proteins per well were loaded on Mini Protean TGX gels (7.5% #4561026 or 12.0% #4561046; Bio-Rad, Hercules, CA, USA).

The Pierce™ BCA Protein Assay Kit (Catalog No. 23227: Thermo Scientific™) was used for accurate determination of protein concentrations. The gel was electrophoresed at 200 V for 30 min and then transferred to a polyvinylidene fluoride (PDVF) membrane (Catalog No. IPVH00010, Merck Millipore, Burlington, USA) at 100 V for 60 min. Diluted primary antibodies were added and incubated at 4℃ overnight, then diluted secondary antibodies were added and incubated for 60 minutes. The secondary antibodies were anti-rabbit IgG, HRP-linked Antibody (1:5,000, #7074, Cell Signaling Technology, Danvers, USA) and anti-mouse IgG, HRP-linked Antibody (1:5,000, #7076, Cell Signaling Technology). The primary antibodies selected for the analysis were anti-c-Jun (1:1,000, #9258, Cell Signaling Technology), anti-phospho-c-Jun (1:1000, #9164, Cell Signaling Technology), anti-HDAC3 (1:2000, #85057, Cell Signaling Technology), anti-fibronectin (1:2000, ab2413, Abcam), and anti-TGF-β1 (1:2000, ab64715, Abcam). The results were quantified by densitometry using ImageJ software, and the level of protein was normalized to that of anti-β-actin (1:10,000, A5441, Sigma-Aldrich, St. Louis, USA) as a loading control. The dilution buffer was Tris Buffered Saline with 0.1% Tween-20 (#9997, Cell Signaling Technology) and 5% Bovine Serum Albumin (#9998, Cell Signaling Technology).

Statistical Analysis

Data are presented as mean ± standard error. For comparison between groups, the Kruskal–Wallis test and Mann–Whitney U test for post–test was used. Spearman's rank correlation analysis was performed to assess the correlation between the histological improvement and the improvements of protein markers related to apoptosis or fibrosis (caspase–3 activity, c–Jun phosphorylation, HDAC3, TGF–β, and fibronectin) in the J, H or J+H groups compared to the I group. A p value < 0.05 was considered statistically significant. Statistical software (SPSS 22.0, Chicago, IL, USA) was used for the statistical analysis.

Figure 2. An animal study design in which 70 of 12-week-old male Sprague-Dawley rats were divided into 5 experimental groups and maintained for 2 weeks.



Results

Effect of combined treatment with JNK inhibitor and HDAC inhibitor on erectile function in nerve-injured rats

Two weeks after injury, the maximal ICP/MAP and AUC/MAP measured in the I group were significantly lower than those in the S group (Figure 3 and Figure 4). In the J and J+H groups, the maximum ICP/MAP and AUC/MAP significantly improved at all voltage stimulations compared to the I group. The H group showed significant increases in erectile responses at 3.0 and 5.0 V stimulation compared to the I group. When comparing the J and H groups, no significant difference was observed in erectile responses. In the J+H group, the max ICP/MAP and AUC/MAP were significantly greater at 1.0 V stimulation compared to the J or H group.

Effect of combined treatment with JNK inhibitor and HDAC inhibitor on structural alterations of the corpus cavernosum in nerve-injured rats

The I group showed decreased immunohistochemical staining for

α-SMA (Figure 5) and a decreased SM/collagen ratio (Figure 6) compared to the S group. The J group showed an increase in both the SM/collagen ratio and immunohistochemical staining for α-SMA, compared to the I group. Compared to the I group, the H group showed an increase in the SM/collagen ratio without an improvement in the immunohistochemical staining for α-SMA. The degree of improvement in the SM/collagen ratio in the J+H group was greater than that in the J and H groups. However, there was no significant difference in the immunohistochemical staining for α-SMA between the J and J+H groups.

Effect of combined treatment with JNK inhibitor and HDAC inhibitor on JNK-driven or HDAC pathways related to cavernosal apoptosis or fibrosis

Regarding cavernosal apoptosis, the I group had a significantly higher caspase-3 activity (Figure 7) and increased phosphorylation of c-Jun (a downstream effector of JNK) (Figure 8) compared to the S group. The J and J+H groups showed an improvement in c-Jun phosphorylation and caspase-3 activity, compared to the I group. There was no difference in c-Jun phosphorylation and caspase-3 activity between the H and I groups.

Regarding cavernosal fibrosis, densitometry showed that the protein expression levels of HDAC3, TGF-β, and fibronectin in the I group were significantly increased compared to those in the S

group (Figure 8). The H and J+H groups showed normalization of the protein expression of HDAC3, TGF-β, and fibronectin. The J group did not show any improvement in the protein expression of HDAC3, TGF-β, or fibronectin compared to the I group.

Correlation between histological improvements and improvements of protein markers related to apoptosis or fibrosis

The Spearman's rank correlation analysis was performed to determine the correlation between the histological improvements (SM/collagen ratio or immunohistochemical staining for α-SMA) and the improvements of protein markers related to apoptosis or fibrosis. Regarding the H and J+H groups, the increase in the SM/collagen ratio compared to the I group was significantly correlated with the decrease in expression of protein markers related to fibrosis (HDAC3, TGF-β and fibronectin) compared to the I group (Table 1). Regarding the J and J+H groups, the increase in the immunohistochemical staining for α-SMA compared to the I group was significantly correlated with the decrease in expression of protein markers related to apoptosis (c-Jun phosphorylation and caspase-3 activity) compared to the I group (Table 1).

Table 1. Spearman's rank correlation coefficient analysis performed to analyze the correlation between histological improvements and improvements of protein markers related to apoptosis or fibrosis. (A) Correlation between changes in smooth muscle/collagen ratio and changes in expression of protein markers related to apoptosis (caspase-3 activity and cJun phosphorylation) or fibrosis (HDAC3, TGF-β and fibronectin) in the treatment groups (H, J and J+H groups) compared to the I group. (B) Correlation between changes in smooth muscle content and changes in expression of protein markers related to apoptosis (caspase-3 activity and cJun phosphorylation) in the treatment groups (H, J and J+H groups) compared to the I group.

(A)

	Smooth muscle/collagen ratio					
	H		J		J+H	
	rho	*p* value	rho	*p* value	rho	*p* value
Caspase-3 activity	0.107	0.819	-0.714	0.071	-0.821	0.023
Phospho-/total c-Jun ratio	0.283	0.460	-0.571	0.139	-0.817	0.007
HDAC3	-0.839	0.001	-0.191	0.574	-0.895	0.000
TGF-β	-0.821	0.023	-0.714	0.071	-0.857	0.014
Fibronectin	-0.886	0.019	-0.200	0.704	-0.943	0.005

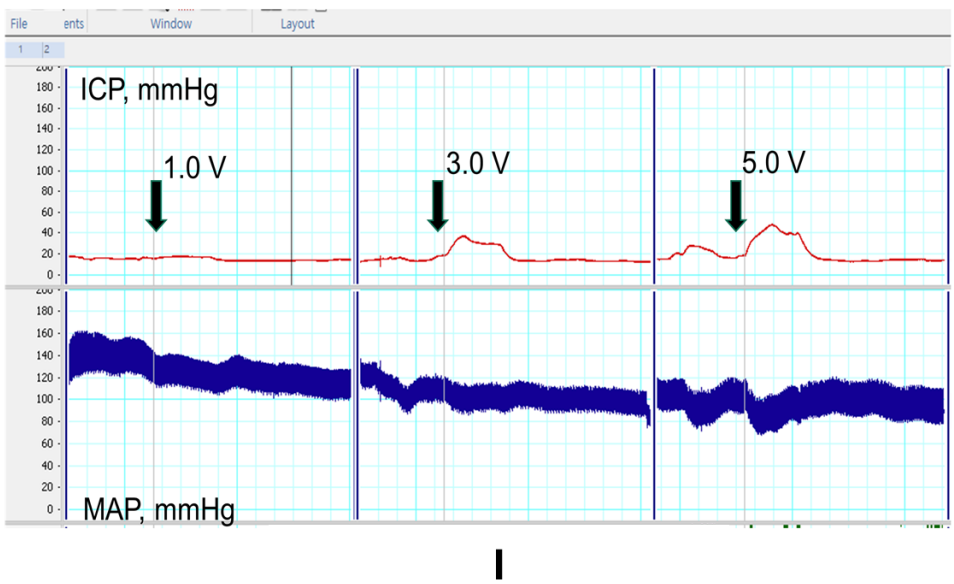
(B)

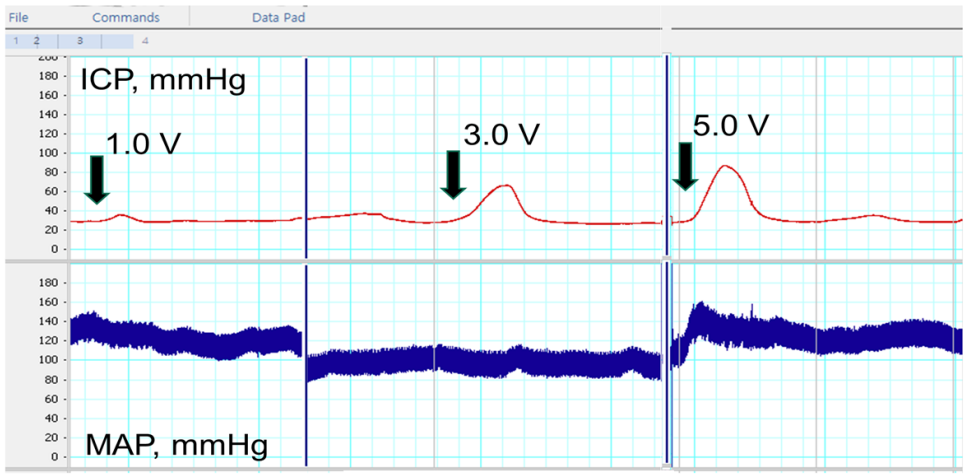
	% Smooth muscle content					
	H		J		J+H	
	rho	*p* value	rho	*p* value	rho	*p* value
Caspase-3 activity	-0.214	0.645	-0.929	0.003	-0.821	0.023
Phospho-/total c-Jun ratio	0.067	0.865	-0.833	0.010	-0.717	0.030

HDAC: histone deacetylase; TGF-β: transforming growth factor-

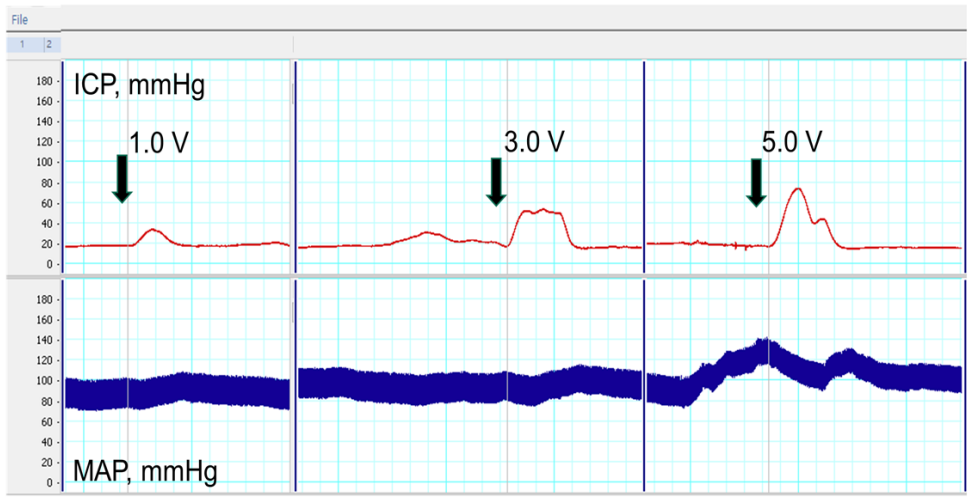
β; CNCI: cavernosal nerve crush injury; HDACi: histone deacetylase inhibitor; JNKi: c-Jun N-terminal kinase inhibitor; I: bilateral CNCI group (n=7); H: bilateral CNCI treated with daily administration of 25mg/kg HDACi (SAHA) group (n=7); J: bilateral CNCI treated with daily intraperitoneal injection of 10 mg/kg JNKi group (n=7); J+H: bilateral CNCI treated with daily combined administration of HDACi and JNKi group (n=7).

Figure 3. Representative ICP/MAP traces for each group show the erectile response to electrostimulation at 2 weeks after CNCI; MAP: mean arterial pressure; ICP: intracavernous pressure; CNCI: cavernosal nerve crush injury; HDACi: histone deacetylase inhibitor; JNKi: c-Jun N-terminal kinase inhibitor; S: sham surgery group (n = 7); I: bilateral CNCI group (n = 7); H: bilateral CNCI treated with daily administration of 25mg/kg HDACi (SAHA) group (n = 7); J: bilateral CNCI treated with daily intraperitoneal injection of 10 mg/kg JNKi group (n = 7); J+H: bilateral CNCI treated with daily combined administration of HDACi and JNKi group (n = 7).

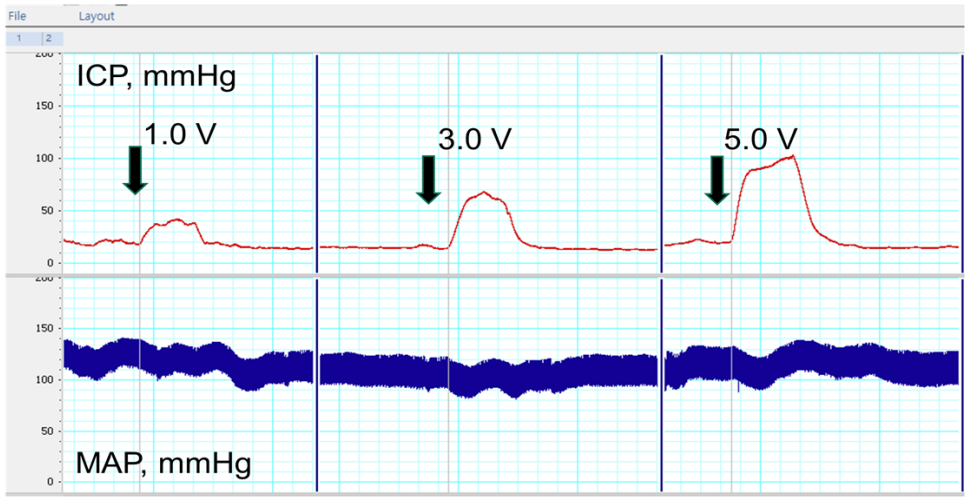



File
Commands
Data Pad
ICP, mmHg
1.0 V
3.0 V
5.0 V
MAP, mmHg

H

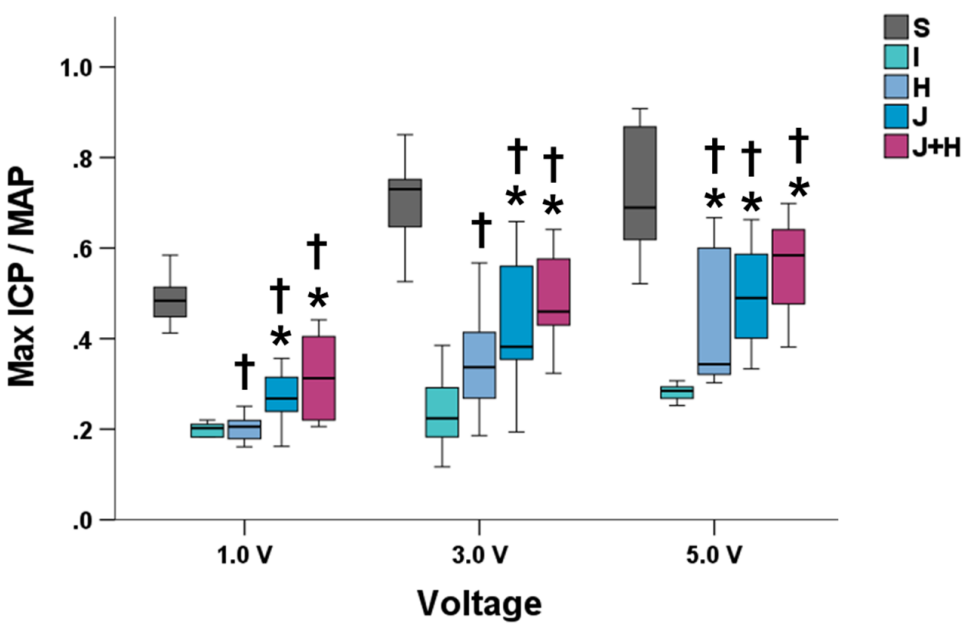

File
ICP, mmHg
1.0 V
3.0 V
5.0 V
MAP, mmHg

J


File
Layout
ICP, mmHg
1.0 V
3.0 V
5.0 V
150
100
50
0
150
100
50
0
MAP, mmHg

J+H

Figure 4. The erectile response of each group to the electrical stimulation at 2 weeks after CNCI were compared. ICP/MAP and AUC/MAP were presented as bar graphs. Asterisk indicates $p<0.05$ vs I group. Dagger indicates $p<0.05$ vs S group. Double dagger indicates $p<0.05$ vs H and J group. AUC: area under the curve; MAP: mean arterial pressure; ICP: intracavernous pressure; CNCI: cavernosal nerve crush injury; HDACi: histone deacetylase inhibitor; JNKi: c-Jun N-terminal kinase inhibitor; S: sham surgery group (n = 7); I: bilateral CNCI group (n = 7); H: bilateral CNCI treated with daily administration of 25mg/kg HDACi (SAHA) group (n = 7); J: bilateral CNCI treated with daily intraperitoneal injection of 10 mg/kg JNKi group (n = 7); J+H: bilateral CNCI treated with daily combined administration of HDACi and JNKi group (n = 7).



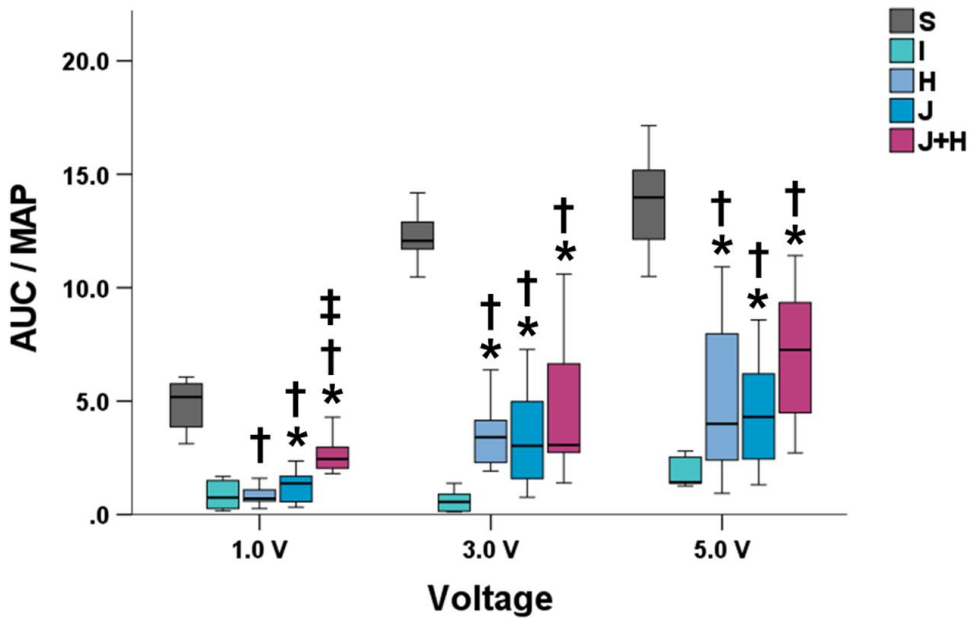
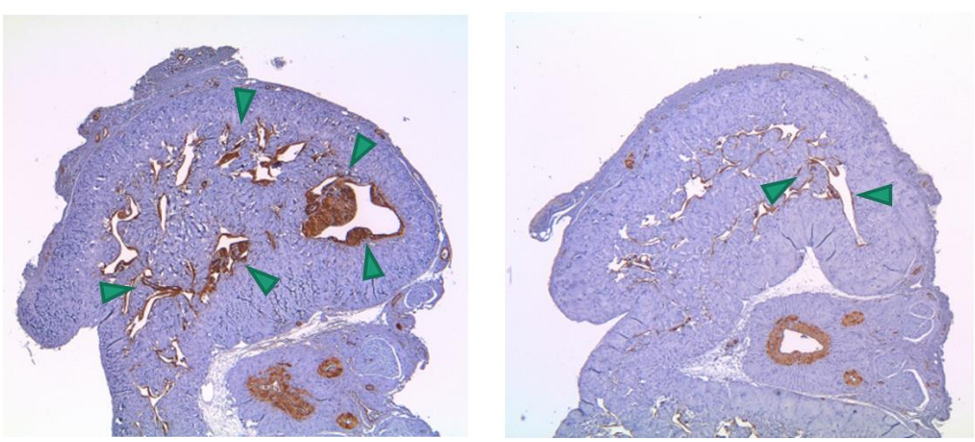
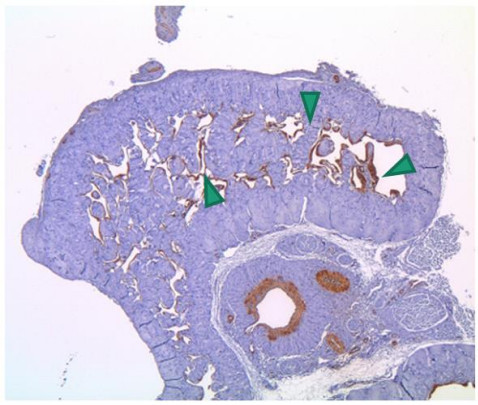
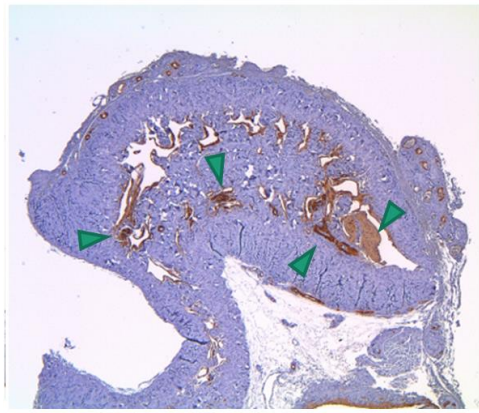

S
I
H
J
J+H
20.0
15.0
10.0
5.0
.0
AUC / MAP
1.0 V
3.0 V
5.0 V
Voltage

Figure 5. Comparison of SM content by detecting α-SMA using immunohistochemical staining at 2 weeks after CNCI. Green arrowheads indicate the smooth muscle. Asterisk indicates $p<0.05$ vs I group. Dagger indicates $p<0.05$ vs S group. Double dagger indicated $p<0.05$ vs H and J group. SM: smooth muscle; SMA = smooth muscle actin; HDACi: histone deacetylase inhibitor; JNKi: c-Jun N-terminal kinase inhibitor; CNCI: cavernosal nerve crush injury; S: sham surgery group (n = 7); I: bilateral CNCI group (n = 7); H: bilateral CNCI treated with daily administration of 25mg/kg HDACi (SAHA) group (n = 7); J: bilateral CNCI treated with daily intraperitoneal injection of 10 mg/kg JNKi group (n = 7); J+H: bilateral CNCI treated with daily combined administration of HDACi and JNKi group (n = 7).

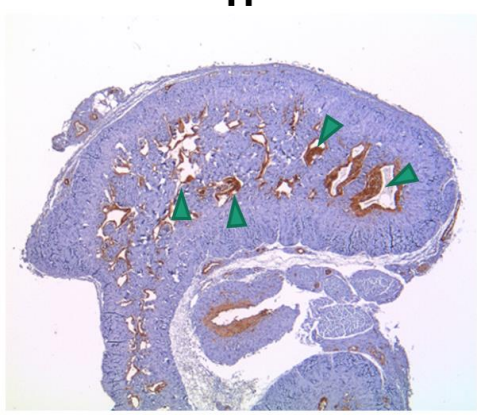




H



J



J+H

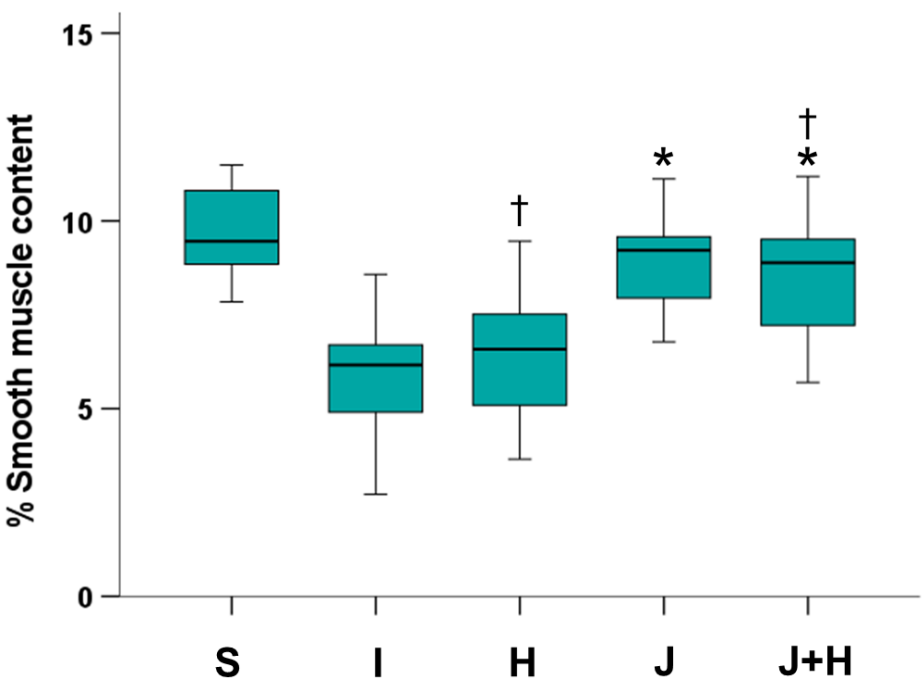
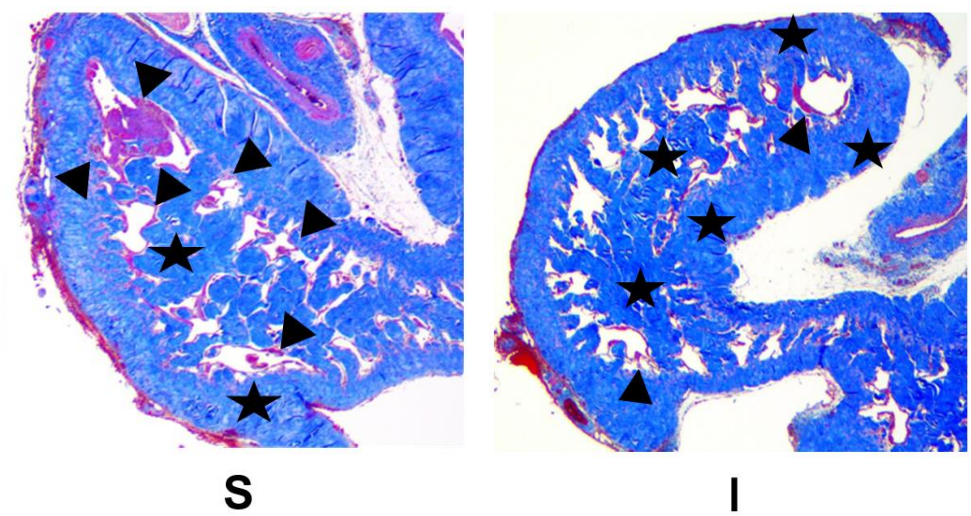
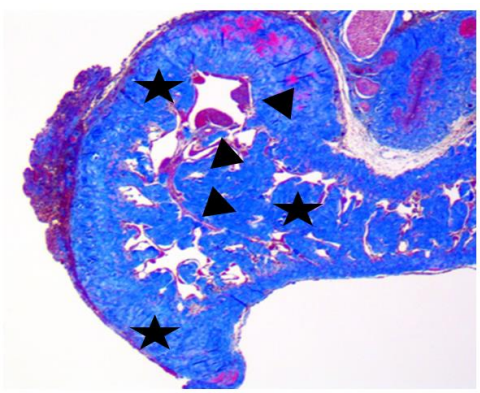
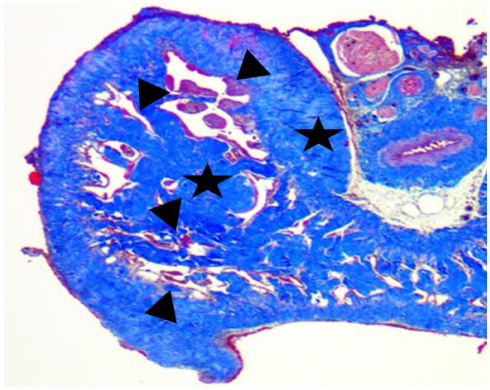


Figure 6. Comparison of SM/collagen ratio using Masson's trichrome staining at 2 weeks after CNCI. Black arrowheads indicate the smooth muscle. Black asterisks indicate collagen deposition in the corpus cavernosum. Asterisk indicates $p<0.05$ vs I group. Dagger indicates $p<0.05$ vs S group. Double dagger indicated $p<0.05$ vs H and J group. SM: smooth muscle; SMA = smooth muscle actin; HDACi: histone deacetylase inhibitor; JNKi: c-Jun N-terminal kinase inhibitor; CNCI: cavernosal nerve crush injury; S: sham surgery group (n = 7); I: bilateral CNCI group (n = 7); H: bilateral CNCI treated with daily administration of 25mg/kg HDACi (SAHA) group (n = 7); J: bilateral CNCI treated with daily intraperitoneal injection of 10 mg/kg JNKi group (n = 7); J+H: bilateral CNCI treated with daily combined administration of HDACi and JNKi group (n = 7).

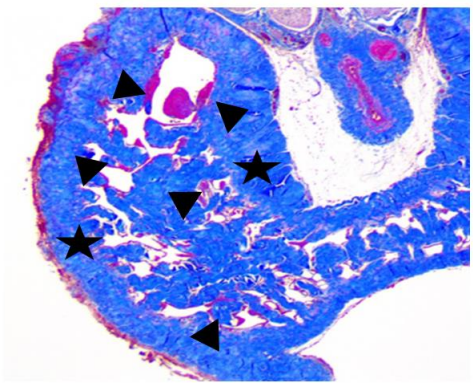




H



J



J+H

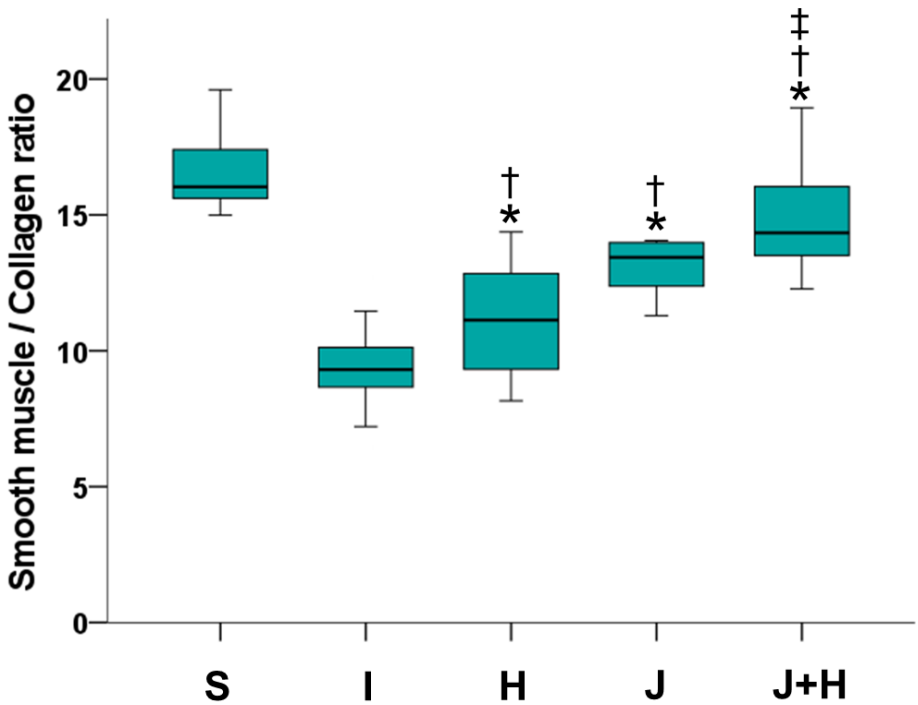


Figure 7. Comparison of caspase-3 activity by presenting the fold change over control using ELISA at 2 weeks after CNCI; asterisk indicates $p<0.05$ vs I group. ELISA: enzyme-linked immunosorbent assay; HDACi: histone deacetylase inhibitor; JNKi: c-Jun N-terminal kinase inhibitor; CNCI: cavernosal nerve crush injury; S: sham surgery group (n = 7); I: bilateral CNCI group (n = 7); H: bilateral CNCI treated with daily administration of 25mg/kg HDACi (SAHA) group (n = 7); J: bilateral CNCI treated with daily intraperitoneal injection of 10 mg/kg JNKi group (n = 7); J+H: bilateral CNCI treated with daily combined administration of HDACi and JNKi group (n = 7).

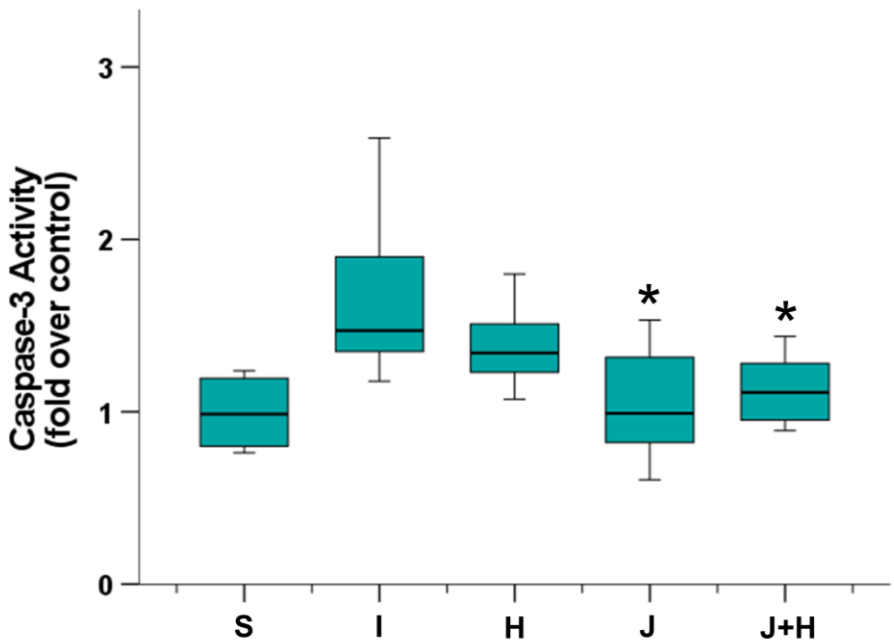
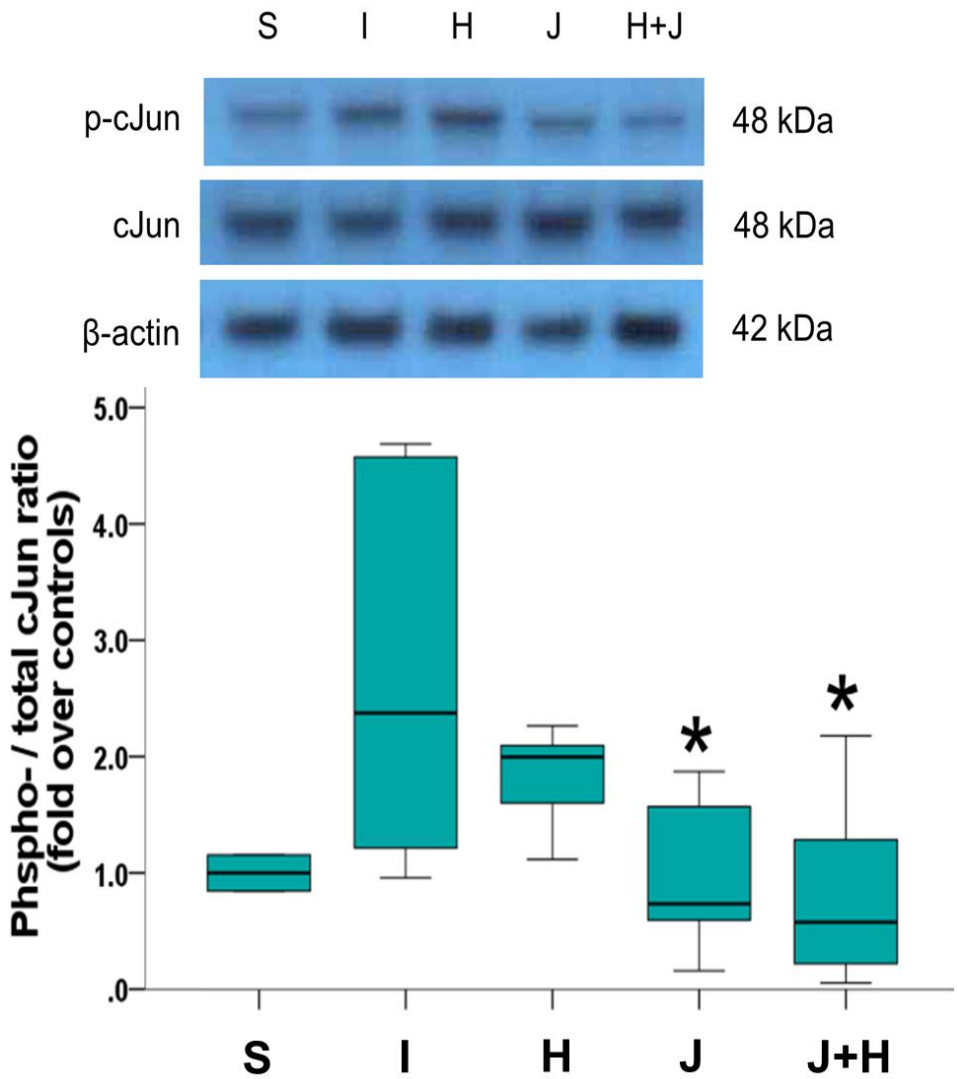
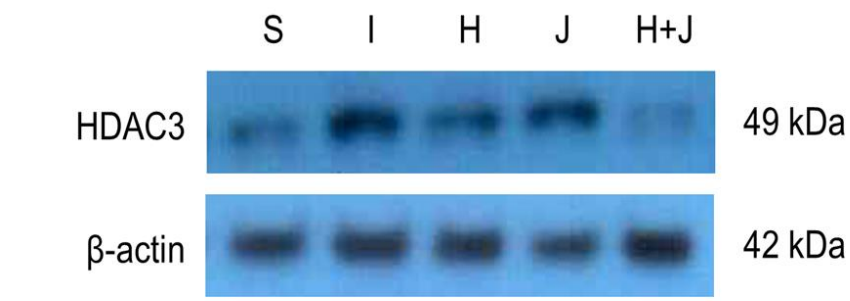


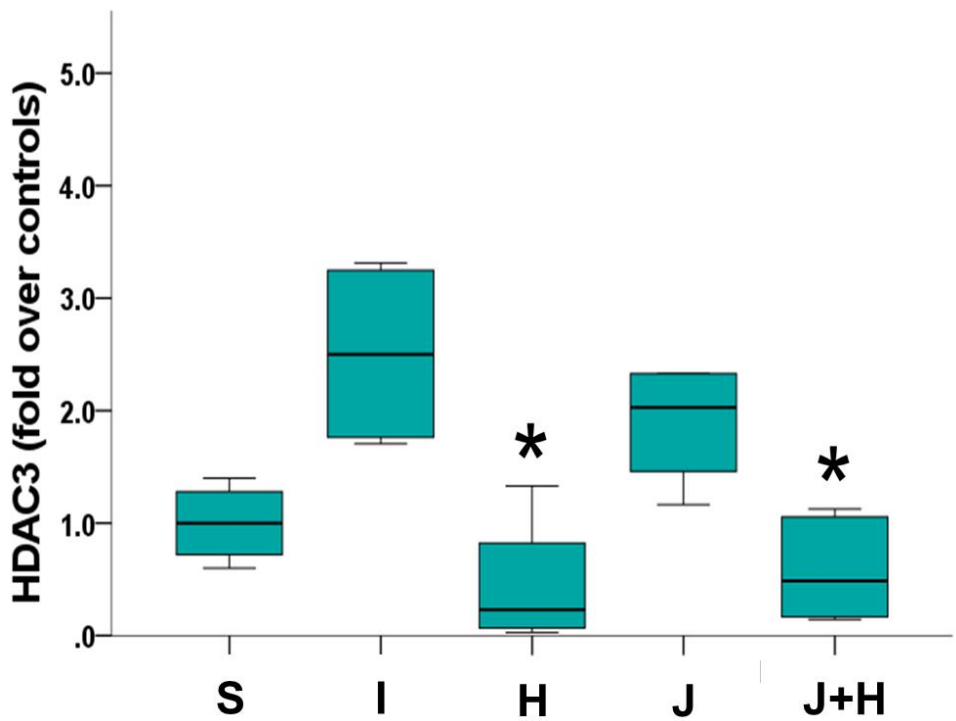
Figure 8. Comparison of the protein expression levels of phosphorylated c-Jun, c-Jun, HDAC3, TGF-β, fibronectin using the western-blot analysis at 2 weeks after CNCI. Each figure was expressed as a bar graph by measuring the protein expression level relative to β-actin using densitometry. (A) bar graph of phosphor-c-JUN/total c-JUN ratio, (B) bar graph of HDAC3 by fold over control, (C) bar graph of TGF-β1 by fold over control, (D) bar graph of fibronectin by fold over control; asterisk indicates $p<0.05$ vs I group. HDACi: histone deacetylase inhibitor; JNKi: c-Jun N-terminal kinase inhibitor; CNCI: cavernosal nerve crush injury; S: sham surgery group (n = 7); I: bilateral CNCI group (n = 7); H: bilateral CNCI treated with daily administration of 25mg/kg HDACi (SAHA) group (n = 7); J: bilateral CNCI treated with daily intraperitoneal injection of 10 mg/kg JNKi group (n = 7); J+H: bilateral CNCI treated with daily combined administration of HDACi and JNKi group (n = 7).

(A)

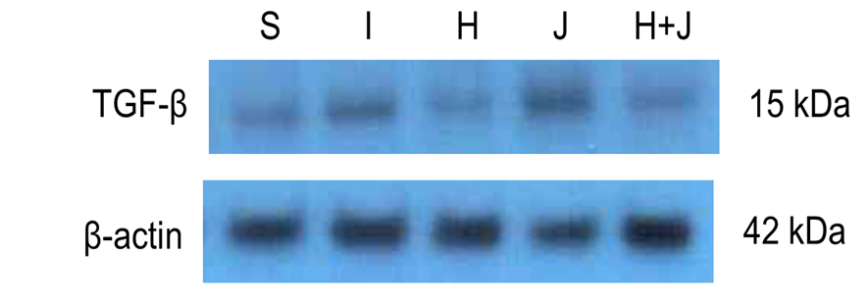

S
I
H
J
H+J
p-cJun
48 kDa
cJun
48 kDa
β-actin
42 kDa
Phspho- / total cJun ratio
(fold over controls)
5.0
4.0
3.0
2.0
1.0
.0
*
*
S
I
H
J
J+H

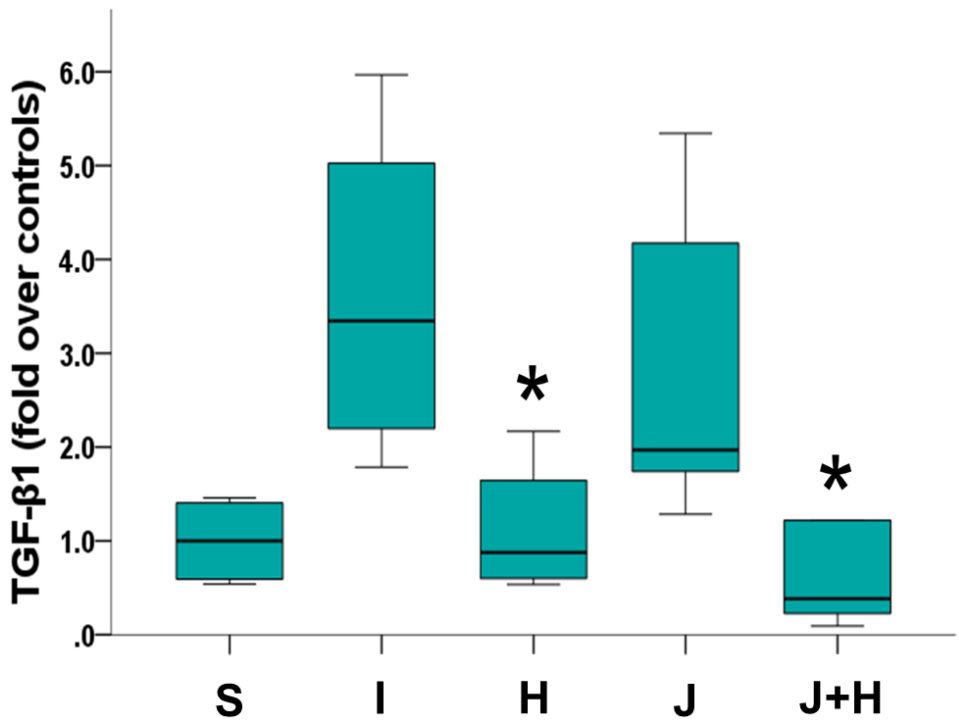
(B)


S
I
H
J
H+J
HDAC3
49 kDa
β-actin
42 kDa

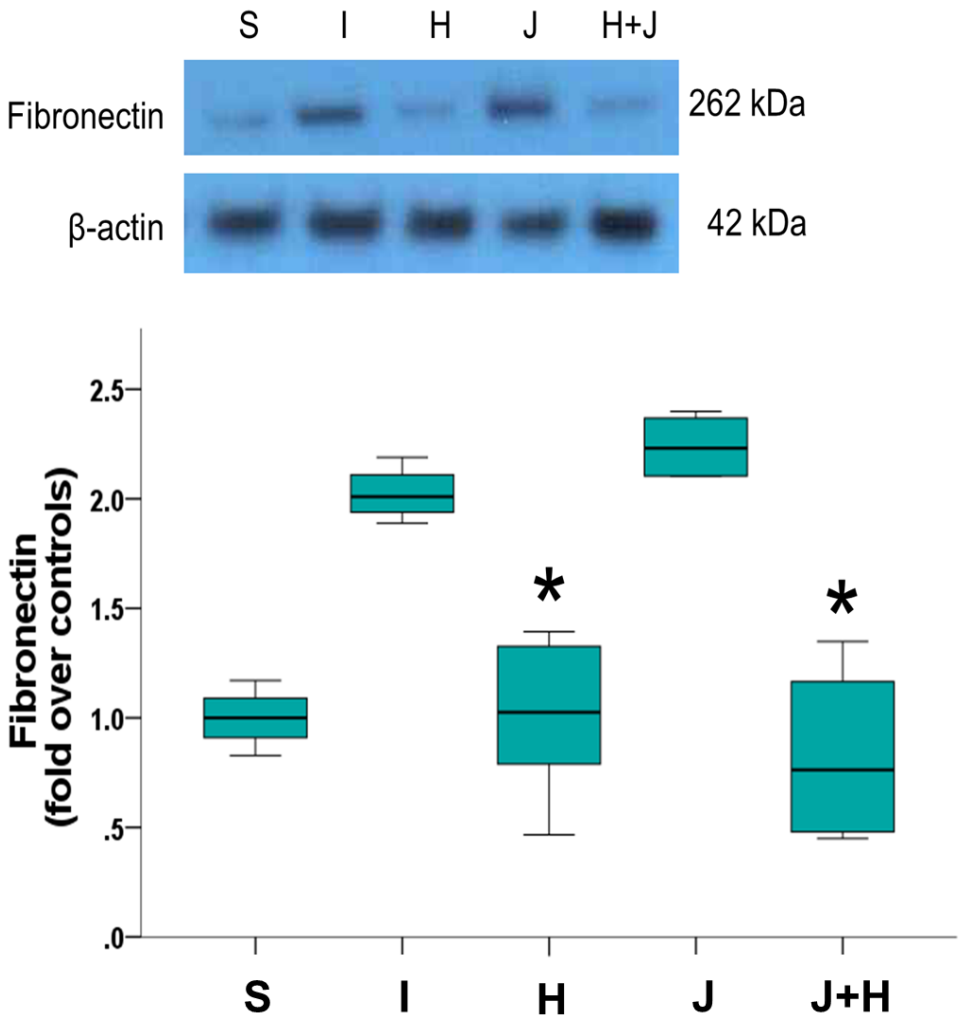

HDAC3 (fold over controls)
5.0
4.0
3.0
2.0
1.0
.0
*
*
S
I
H
J
J+H

(C)


S
I
H
J
H+J
TGF-β
15 kDa
β-actin
42 kDa


TGF-β1 (fold over controls)
6.0
5.0
4.0
3.0
2.0
1.0
.0
*
*
S
I
H
J
J+H

(D)


S
I
H
J
H+J
Fibronectin
262 kDa
β-actin
42 kDa
Fibronectin
(fold over controls)
2.5
2.0
1.5
1.0
.5
.0
*
*
S
I
H
J
J+H

Discussion

Main findings of this study

CVOD caused by cavernosal apoptosis and fibrosis is one of the most important causes of post-RP ED (Capogrosso et al., 2016; Mulhall, 2008). In the post-injury phase, apoptosis occurs most actively within 1 week of injury and then gradually decreases. Fibrosis has been found to initiate from 1 week after injury and accumulates continuously (T. B. Kim, Cho, Paik, & Kim, 2012; User et al., 2003). Our previous studies reported incomplete restoration of erectile response to electrostimulation and cavernosal veno-occlusive function by inhibiting both JNK and LIMK2 in a rat model of CNCI (Cho, Lee, Park, & Kim, 2020; S. W. Kim et al., 2019). Therefore, we focused on HDAC, which is known to regulate TGF-β as a key molecule in fibrosis. In a recent study, we showed that the administration of HDACi significantly improved CVOD in a rat model of CNCI (Cho, Lee, Son, et al., 2020). Thus, the aim of this study was to assess the therapeutic effect of combined administration of JNKi and HDACi on erectile function by suppressing apoptosis and fibrosis in a rat model of CNCI. The main findings identified in this study are as follows:

1) Compared with the single agent treatment group, the

combination treatment may have an additive therapeutic effect on the improvement of erectile function and SM/collagen ratio in the corpus cavernosum. In particular, the improvement of the additional erectile responses was greater when stimulating with the low voltage than the high voltage in the combination treatment group.

2) Inhibition of the JNK and HDAC pathways immediately after nerve injury confirmed the rectification of apoptosis and fibrosis of the corpus cavernosum. HDCAi (SAHA) showed anti-fibrotic effects by suppressing the production of fibronectin and increasing the SM/collagen ratio. JNKi showed anti-apoptotic effects by inhibiting the phosphorylation of c-Jun and increasing the SM content.

The HDAC pathway and the role of HDAC inhibitors in corporal fibrosis

HDAC removes the acetyl group from the hyperacetylated histone tail, resulting in chromatin compaction as a strong bond between histones and DNA (Li & Sun, 2019). This action consequently regulates the inhibition of transcriptional activity. In a previous study, 18 HDACs were identified and classified into four classes (Class I: HDAC1, 2, 3, and 8; Class II: HDAC4, 5, 6, 7, 9, 10, Class III: SIRT1-7, and Class IV: HDAC11), but the differences in their roles have not been clearly identified. SAHA, the HDACi we used in

this study, inhibits both class I and II of HDAC (Eckschlager, Plch, Stiborova, & Hrabeta, 2017). Recently, the anti-fibrosis effect of HDACi has been highlighted, and the mechanism in human fibrosis-associated diseases of the liver, heart, lung, kidney, etc. is being studied (Tang, Yan, & Zhuang, 2013). Hannan et al. reported that treatment with valproic acid (HDACi) in a rat model of bilateral CNCI for 2 and 4 weeks improved cavernosal fibrosis and recovered erectile response (Hannan et al., 2014). In the present study, a significant increase in the SM/collagen ratio was found in the HDACi-treated group compared to that in the injury group. The H and J+H groups showed that the protein expression of fibronectin, HDAC3, and TGF-β improved to meet the level of the sham control. Furthermore, the improvement of SM/collagen ratios in the HDACi-treated rats (the H and J+H groups) compared to the nerve-injured rats (the I group) was significantly correlated with the improvement of expression of protein markers related to fibrosis in the HDACi-treated rats compared to the nerve-injured rats. In addition, a reduction in TGF-β by HDACi administration occurred due to a mechanism in which TGF-β is regulated as a downstream molecule of HDAC.

Inhibition of HDAC has also been suggested to be effective in another fibrotic disease. Peyronie's disease is known as one of the diseases in which progressive chronic fibrotic plaques on the tunica albuginea of the penis is the main pathophysiology. Fibrotic plaques are achieved by locally excessive accumulation of extracellular

matrix by transdifferentiating fibroblasts into myofibroblasts. Ryu et al. studied the effect of alleviating the fibrosis reaction when HDAC2 was inhibited in fibroblasts obtained from human Peyronie's disease plaques (Ryu et al., 2013). They reported that knockdown of HDAC2 in fibroblasts improved excessive extracellular matrix accumulation by a mechanism that inhibits the TGF-β1 induced Smad2/3 signaling pathway.

The JNK pathway and the role of JNK inhibitors in corporal apoptosis

JNK activates intrinsic pathways related to mitochondrial processes of c-Jun to proceed with apoptosis (Dhanasekaran & Reddy, 2008). JNKi improves apoptosis in cavernosal tissues, such as SM, by inhibiting c-Jun phosphorylation. Our previous study reported improvement in erectile function through an anti-apoptotic effect after 2 weeks of JNKi administration in a rat model of CNCI (J. Park, Chai, et al., 2018). In this study, α-SMA content and Caspase-3 activity recovered to the level of the sham control. In addition, a significant increase in the cavernosal SM/collagen ratio was observed in the J group, which was higher than that in the H group. This is the result of improving the content of cavernosal SM through inhibition of the apoptotic pathway by JNKi. Furthermore, the improvement of SM content in the JNKi-treated rats (the J and J+H groups) compared to the nerve-injured rats (the I group) was

significantly correlated with the improvement of expression of protein markers related to apoptosis in the JNKi-treated rats compared to the nerve-injured rats. Meanwhile, no improvement in protein level of TGF-β or fibronectin was observed in the JNKi-treated group.

Diabetic erectile dysfunction is known to be caused by high blood glucose, which increases oxidative stress and damage the corpus cavernosal endothelium and nerves. This damage is considered to be due to increased apoptosis and decreased self-recovery of cells in diabetes. Liu et al. described that inhibition of JNK reduced autophagy-induced apoptosis by rectifying JNK/Bcl-2 pathway of transplanted mesenchymal stem cells in a diabetic ED mouse model (G. Y. Liu et al., 2015).

Combination therapy of JNK inhibitor and HDAC inhibitor for restoration of erectile function

According to a previous report, the ICP also increased as the voltage stimulation increased in the electrostimulation test; 2.0 V stimulation might be an intensity that exceeded the threshold sufficiently, and when the voltage exceeded 10 V, tissue damage was observed (Zhao et al., 2018). However, for the electrostimulation test, the ideal stimulation voltage level has not yet been determined. The combination treatment of HDACi and JNKi showed additive improvement of ICP at 1.0 V stimulation

compared to the single agent treatment group in this study. The erectile response to electrostimulation responded sensitively at a low 1.0 V stimulation, which is a level that barely exceeds the threshold, but the recovered tissue response may have been masked at a strong voltage sufficiently exceeding the threshold of 3.0 and 5.0 V stimulation. The acute phase, two weeks after injury, is the time when apoptosis has a greater effect on tissue degeneration than fibrosis. Therefore, the low ICP improvement of the H group may be due to the anti-fibrotic effect of HDACi rather than an anti-apoptotic effect on cavernosal tissue during the acute phase.

Limitations of this study

When combining HDACi and JNKi, histological changes such as apoptosis and fibrosis and erectile function did not completely recover to the level of the sham control. First, two weeks of drug administration may not have been sufficient to confirm the therapeutic effects on fibrosis and apoptosis. As mentioned above, the fibrosis reaction increases and steadily accumulates 1 week after injury. Apoptosis is maximally activated 1 week after CN injury and persists for up to 4-5 weeks (Ferrini et al., 2009; T. B. Kim et al., 2012). Therefore, it is necessary to further investigate the recovery of erectile response, fibrosis, and apoptosis through chronic treatment with HDACi and JNKi. Second, SAHA, a pan-

HDACi, might cause several adverse effects, such as cytotoxicity, by acting on normal cells (Xu, Parmigiani, & Marks, 2007). However, selective HDACis such as mocetinostat, abexinostat, and fimepinostat are modestly potent inhibitors of HDAC2 or 3. Therefore, the present study used SAHA because we wanted the potent inhibition of HDAC2 or HDAC3, which contributed to tissue fibrogenesis. The results of further research using selective HDACis that inhibit HDAC2 and HDAC3 will address this limitation. Nevertheless, in this study, HDAC3 of class I was distributed in cavernosal tissue, and the anti-fibrotic effect was confirmed to restore fibronectin and the SM/collagen ratio. Third, since this was an animal study using the young rats of 12-week-old, most of the rats would have normal erectile function before surgery. In addition, the effects of drugs may not be the same in the human disease group due to biological differences, but it is helpful to proactively study the mechanisms and effects of the administered substances. The combination therapy of HDACi and JNKi may have the potential to be a mechanism-specific targeted therapy for post-RP ED. In the future, it will be the basis for discovering other molecular targets and the appropriate treatment duration.

Conclusions

Our data indicate that the combined administration of HDACi and JNKi during the acute phase after CNCI may preserve erectile function by inhibiting apoptosis and fibrosis in a rat model of ED induced by bilateral CNCI. These effects appear to be attributed to normalization of the JNK/cJun pathway related to apoptosis and the HDAC3/TGF-β signaling related to fibrosis. In addition, there appears likely to be an additive improvement of the erectile function, particularly at low stimulation and the cavernosal SM/collagen ratio due to combination therapy. Combined inhibition of JNK and HDAC might be a potential treatment option for mechanism-specific targeted therapy of post-RP ED. Further studies are needed to provide increased knowledge regarding post-RP ED.

References

Capogrosso, P., Salonia, A., Briganti, A., & Montorsi, F. (2016). Postprostatectomy Erectile Dysfunction: A Review. *World J Mens Health, 34*(2), 73-88. doi:10.5534/wjmh.2016.34.2.73

Cho, M. C., Lee, J., Park, J., & Kim, S. W. (2020). Restoration of Cavernous Veno-Occlusive Function through Chronic Administration of a Jun-Amino Terminal Kinase Inhibitor and a LIM-Kinase 2 Inhibitor by Suppressing Cavernous Apoptosis and Fibrosis in a Rat Model of Cavernous Nerve Injury: A Comparison with a Phosphodiesterase Type 5 Inhibitor. *World J Mens Health*. doi:10.5534/wjmh.200085

Cho, M. C., Lee, J., Son, H., & Kim, S. W. (2020). Rectification of cavernosal fibrosis and veno-occlusive dysfunction by administration of suberoylanilide hydroxamic acid in a rat model of cavernosal nerve injury: Comparison with a PDE5 inhibitor. *Andrology*. doi:10.1111/andr.12922

Cohen, D. J., & Glina, S. (2015). Penile rehabilitation after radical prostatectomy. *Curr Drug Targets, 16*(5), 451-458. doi:10.2174/1389450116666150202153832

Dhanasekaran, D. N., & Reddy, E. P. (2008). JNK signaling in apoptosis. *Oncogene, 27*(48), 6245-6251. doi:10.1038/onc.2008.301

Dorin, R. P., Daneshmand, S., Lassoff, M. A., Cai, J., Skinner, D. G., & Lieskovsky, G. (2012). Long-term outcomes of open radical retropubic prostatectomy for clinically localized prostate cancer in the prostate-specific antigen era. *Urology, 79*(3), 626-631. doi:10.1016/j.urology.2011.09.051

Eckschlager, T., Plch, J., Stiborova, M., & Hrabeta, J. (2017). Histone Deacetylase Inhibitors as Anticancer Drugs. *Int J Mol Sci, 18*(7). doi:10.3390/ijms18071414

Ferrini, M. G., Kovanecz, I., Sanchez, S., Umeh, C., Rajfer, J., & Gonzalez-Cadavid, N. F. (2009). Fibrosis and loss of smooth muscle in the corpora cavernosa precede corporal veno-occlusive dysfunction (CVOD) induced by experimental cavernosal nerve damage in the rat. *J Sex Med, 6*(2), 415-428. doi:10.1111/j.1743-6109.2008.01105.x

Ficarra, V., Novara, G., Fracalanza, S., D'Elia, C., Secco, S., Iafrate, M., . . . Artibani, W. (2009). A prospective, non-randomized trial comparing robot-assisted laparoscopic and retropubic radical prostatectomy in one European institution. *BJU Int, 104*(4), 534-539. doi:10.1111/j.1464-410X.2009.08419.x

Fode, M., Ohl, D. A., Ralph, D., & Sonksen, J. (2013). Penile rehabilitation after radical prostatectomy: what the evidence really says. *BJU Int, 112*(7), 998-1008. doi:10.1111/bju.12228

Glenisson, W., Castronovo, V., & Waltregny, D. (2007). Histone deacetylase 4 is required for TGFbeta1-induced myofibroblastic differentiation. *Biochim Biophys Acta, 1773*(10), 1572-1582. doi:10.1016/j.bbamcr.2007.05.016

Global Burden of Disease Cancer, C., Fitzmaurice, C., Abate, D., Abbasi, N., Abbastabar, H., Abd-Allah, F., . . . Murray, C. J. L. (2019). Global, Regional, and National Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life-Years for 29 Cancer Groups, 1990 to 2017: A Systematic Analysis for the Global Burden of Disease Study. *JAMA Oncol*. doi:10.1001/jamaoncol.2019.2996

Hannan, J. L., Kutlu, O., Stopak, B. L., Liu, X., Castiglione, F., Hedlund, P., . . . Bivalacqua, T. J. (2014). Valproic acid prevents penile fibrosis and erectile dysfunction in cavernous nerve-injured rats. *J Sex Med, 11*(6), 1442-1451. doi:10.1111/jsm.12522

Kim, S. W., Lee, J., Park, J., Chai, J. S., Oh, S., Paick, J. S., & Cho, M. C. (2019). Combination of LIM-kinase 2 and Jun Amino-terminal Kinase Inhibitors Improves Erectile Function in a Rat Model of Cavernous Nerve Injury. *Urology, 131*, 136-143. doi:10.1016/j.urology.2019.06.005

Kim, T. B., Cho, M. C., Paik, J. S., & Kim, S. W. (2012). Is It Possible to Recover Erectile Function Spontaneously after Cavernous Nerve Injury? Time-Dependent Structural and

Functional Changes in Corpus Cavernosum Following Cavernous Nerve Injury in Rats. *World J Mens Health, 30*(1), 31-39.

Lepor, H. (2005). A review of surgical techniques for radical prostatectomy. *Rev Urol, 7 Suppl 2*, S11-17. Retrieved from https://www.ncbi.nlm.nih.gov/pubmed/16985892

Lepor, H., Gregerman, M., Crosby, R., Mostofi, F. K., & Walsh, P. C. (1985). Precise localization of the autonomic nerves from the pelvic plexus to the corpora cavernosa: a detailed anatomical study of the adult male pelvis. *J Urol, 133*(2), 207-212. doi:10.1016/s0022-5347(17)48885-9

Li, W., & Sun, Z. (2019). Mechanism of Action for HDAC Inhibitors-Insights from Omics Approaches. *Int J Mol Sci, 20*(7). doi:10.3390/ijms20071616

Liu, G. Y., Jiang, X. X., Zhu, X., He, W. Y., Kuang, Y. L., Ren, K., . . . Gou, X. (2015). ROS activates JNK-mediated autophagy to counteract apoptosis in mouse mesenchymal stem cells in vitro. *Acta Pharmacol Sin, 36*(12), 1473-1479. doi:10.1038/aps.2015.101

Liu, L., Fang, Y. Q., Xue, Z. F., He, Y. P., Fang, R. M., & Li, L. (2012). Beta-asarone attenuates ischemia-reperfusion-induced autophagy in rat brains via modulating JNK, p-JNK, Bcl-2 and Beclin 1. *Eur J Pharmacol, 680*(1-3), 34-40. doi:10.1016/j.ejphar.2012.01.016

Meyer, J. P., Gillatt, D. A., Lockyer, R., & Macdonagh, R. (2003). The effect of erectile dysfunction on the quality of life of men after radical prostatectomy. *BJU Int, 92*(9), 929-931. doi:10.1111/j.1464-410x.2003.04530.x

Mohler, J. L., Antonarakis, E. S., Armstrong, A. J., D'Amico, A. V., Davis, B. J., Dorff, T., . . . Freedman-Cass, D. A. (2019). Prostate Cancer, Version 2.2019, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw, 17*(5), 479-505. doi:10.6004/jnccn.2019.0023

Mulhall, J. P. (2008). Penile rehabilitation following radical prostatectomy. *Curr Opin Urol, 18*(6), 613-620. doi:10.1097/MOU.0b013e3283136462

Park, J., Chai, J. S., Kim, S. W., Paick, J. S., & Cho, M. C. (2018). Inhibition of Jun N-terminal Kinase Improves Erectile Function by Alleviation of Cavernosal Apoptosis in a Rat Model of Cavernous Nerve Injury. *Urology, 113*, 253 e259-253 e216. doi:10.1016/j.urology.2017.11.040

Park, J., Cho, S. Y., Park, K., Chai, J. S., Son, H., Kim, S. W., . . . Cho, M. C. (2018). Role of inhibiting LIM-kinase2 in improving erectile function through suppression of corporal fibrosis in a rat model of cavernous nerve injury. *Asian J Androl, 20*(4), 372-378. doi:10.4103/aja.aja_82_17

Park, K., Ryu, K. S., Li, W. J., Kim, S. W., & Paick, J. S. (2008).

Chronic treatment with a type 5 phosphodiesterase inhibitor suppresses apoptosis of corporal smooth muscle by potentiating Akt signalling in a rat model of diabetic erectile dysfunction. *Eur Urol, 53*(6), 1282-1288. doi:10.1016/j.eururo.2008.01.032

Philippou, Y. A., Jung, J. H., Steggall, M. J., O'Driscoll, S. T., Bakker, C. J., Bodie, J. A., & Dahm, P. (2018). Penile rehabilitation for postprostatectomy erectile dysfunction. *Cochrane Database Syst Rev, 10*, CD012414. doi:10.1002/14651858.CD012414.pub2

Resnick, M. J., Koyama, T., Fan, K. H., Albertsen, P. C., Goodman, M., Hamilton, A. S., . . . Penson, D. F. (2013). Long-term functional outcomes after treatment for localized prostate cancer. *N Engl J Med, 368*(5), 436-445. doi:10.1056/NEJMoa1209978

Ryu, J. K., Kim, W. J., Choi, M. J., Park, J. M., Song, K. M., Kwon, M. H., . . . Suh, J. K. (2013). Inhibition of histone deacetylase 2 mitigates profibrotic TGF-beta1 responses in fibroblasts derived from Peyronie's plaque. *Asian J Androl, 15*(5), 640-645. doi:10.1038/aja.2013.61

Salonia, A., Adaikan, G., Buvat, J., Carrier, S., El-Meliegy, A., Hatzimouratidis, K., . . . Khera, M. (2017a). Sexual Rehabilitation After Treatment for Prostate Cancer-Part 1: Recommendations From the Fourth International Consultation for Sexual Medicine (ICSM 2015). *J Sex Med, 14*(3), 285-

296. doi:10.1016/j.jsxm.2016.11.325

Salonia, A., Adaikan, G., Buvat, J., Carrier, S., El-Meliegy, A., Hatzimouratidis, K., . . . Khera, M. (2017b). Sexual Rehabilitation After Treatment For Prostate Cancer-Part 2: Recommendations From the Fourth International Consultation for Sexual Medicine (ICSM 2015). *J Sex Med, 14*(3), 297-315. doi:10.1016/j.jsxm.2016.11.324

Sari Motlagh, R., Abufaraj, M., Yang, L., Mori, K., Pradere, B., Laukhtina, E., . . . Shariat, S. F. (2021). Penile Rehabilitation Strategy after Nerve Sparing Radical Prostatectomy: A Systematic Review and Network Meta-Analysis of Randomized Trials. *J Urol, 205*(4), 1018-1030. doi:10.1097/JU.0000000000001584

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA Cancer J Clin, 70*(1), 7-30. doi:10.3322/caac.21590

Singer, P. A., Tasch, E. S., Stocking, C., Rubin, S., Siegler, M., & Weichselbaum, R. (1991). Sex or survival: trade-offs between quality and quantity of life. *J Clin Oncol, 9*(2), 328-334. doi:10.1200/JCO.1991.9.2.328

Tang, J., Yan, H., & Zhuang, S. (2013). Histone deacetylases as targets for treatment of multiple diseases. *Clin Sci (Lond), 124*(11), 651-662. doi:10.1042/CS20120504

User, H. M., Hairston, J. H., Zelner, D. J., McKenna, K. E., & McVary, K. T. (2003). Penile weight and cell subtype specific changes in a post-radical prostatectomy model of erectile dysfunction. *J Urol, 169*(3), 1175-1179. doi:10.1097/01.ju.0000048974.47461.50

Walsh, P. C., Lepor, H., & Eggleston, J. C. (1983). Radical prostatectomy with preservation of sexual function: anatomical and pathological considerations. *Prostate, 4*(5), 473-485. doi:10.1002/pros.2990040506

Wang, X., Wang, X., Liu, T., He, Q., Wang, Y., & Zhang, X. (2014). Systematic review and meta-analysis of the use of phosphodiesterase type 5 inhibitors for treatment of erectile dysfunction following bilateral nerve-sparing radical prostatectomy. *PLoS One, 9*(3), e91327. doi:10.1371/journal.pone.0091327

Xu, W. S., Parmigiani, R. B., & Marks, P. A. (2007). Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene, 26*(37), 5541-5552. doi:10.1038/sj.onc.1210620

Zhao, S., Kang, R., Deng, T., Luo, L., Wang, J., Li, E., . . . Zhao, Z. (2018). Comparison of two cannulation methods for assessment of intracavernosal pressure in a rat model. *PLoS One, 13*(2), e0193543. doi:10.1371/journal.pone.0193543

Abstract

Restoring erectile function by combined treatment with JNK inhibitor and HDAC inhibitor in a rat model of cavernous nerve injury

Junghoon Lee

Urology, College of Medicine

The Graduate School

Seoul National University

Introduction

The main pathophysiologic conditions of erectile dysfunction after radical prostatectomy are considered to be corporal fibrosis and apoptosis induced by cavernosal nerve (CN) injury. In a rat model of CN crush injury (CNCI), we investigated whether combination treatment with JNK inhibitor (JNKi), SP600125, and HDAC inhibitor (HDACi), suberoylanilide-hydroxamic-7 acid (SAHA), for 2 weeks after CNCI would restore erectile function by suppressing fibrosis and apoptosis through normalization of JNK and HDAC pathways.

Materials and methods

Seventy 12-week-old rats were randomly divided into five groups: Sham surgery, CNCI alone, CNCI treated with daily intraperitoneal injection of 10mg/kg JNKi, CNCI treated with daily oral administration of 25.0mg/kg HDACi, and CNCI daily treated with a combination. Two weeks after CNCI, we investigated the erectile response to electrostimulation and conducted histological staining, caspase-3 activity assay, and western blot analysis.

Results

CNCI alone resulted in significantly reduced intracavernosal pressure (ICP)/mean arterial pressure (MAP) and area under the curve/MAP, decreased smooth muscle (SM)/collagen ratio and SM content, higher caspase-3 activity, and increased protein levels of total HDAC3, TGF-β, fibronectin, and c-Jun phosphorylation, compared with the Sham surgery. The CNCI groups exposed to JNKi, HDACi or both showed improvements in erectile-responses and SM/collagen ratio, compared to the CNCI alone. The combined treatment showed additional improvement in erectile responses at 1.0V stimulation and in SM/collagen ratio compared to the single agent treatment. SM content, caspase-3 activity and c-Jun phosphorylation improved in the two CNCI groups exposed to JNKi. The two CNCI groups exposed to HDACi showed normalization of protein levels of HDAC3, fibronectin and TGF-β.

Conclusions

The combined administration of JNKi and HDACi during the acute phase after CNCI in rats can preserve erectile dysfunction by suppressing cavernosal fibrosis and apoptosis by normalizing the HDAC/TGF-β and JNK pathways.

Keywords: apoptosis, fibrosis, erectile dysfunction, histone deacetylase, Jun-amino terminal kinase, prostatectomy

Student number: 2019-34092

*A significant part of this work was published in *Andrology* journal (2022;1-9, DOI:10.1111/andr.13154).