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Amelioration of Cavernosal Fibrosis and Erectile Function by Lysyl Oxidase Inhibition in a Rat Model of Cavernous Nerve Injury

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ABSTRACT

Background: Cavernous nerve injury (CNI) causes fibrosis and loss of smooth muscle cells (SMCs) in the corpus cavernosum and leads to erectile dysfunction, and lysyl oxidase (LOX) activation has been found to play an important role in fibrotic diseases.

Aim: To evaluate the role of LOX in penile fibrosis after bilateral CNI (BCNI).

Methods: Rats underwent BCNI or a sham operation and were treated with vehicle or β-aminopropionitrile, a specific LOX activity inhibitor. 30 days after BCNI, rats were tested for erectile function before penile tissue harvest. LOX and extracellular matrix component expression levels in the corpus cavernosum, including matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), fibronectin (FN), collagen (COL) I, and COL IV, were evaluated by real-time quantitative polymerase chain reaction and western blot. Corporal fibrosis was evaluated by Masson trichrome staining. Localization of LOX and SMC content in the corpus cavernosum were assessed by immunohistochemistry.

Outcomes: Ratio of intracavernous pressure to mean arterial blood pressure; LOX, MMPs, TIMPs, COL I, COL IV, and FN expression; penile fibrosis; penile SMC content.

Results: After BCNI, there was an increase in penile LOX expression and activity, increased penile fibrosis, decreased SMC content, and impaired erectile function. TIMP1, TIMP2, COL I, COL IV, and FN expression was markedly upregulated, whereas the enzyme activity of MMPs was decreased after BCNI. β-Aminopropionitrile treatment, at least in part, prevented a decrease in the ratio of intracavernous pressure to mean arterial blood pressure, decreased penile expression of TIMP1, TIMP2, COL I, COL IV, and FN, increased MMP activity, prevented corporal fibrosis, and preserved SMC content.

Clinical Translation: LOX over-activation contributes to penile fibrosis and LOX inhibition could be a promising strategy in preventing the progression of CNI-induced erectile dysfunction.

Strengths and Limitations: This is the 1st study to demonstrate the role of LOX activation in penile fibrosis. However, the exact mechanism of how LOX influences extracellular matrix protein synthesis and SMC content preservation awaits further investigation.

Conclusion: CNI induced LOX over-activation in cavernous tissue, and inhibition of LOX preserved penile morphology and improved erectile function in a rat model of BCNI. **Wan Z-H, Li G-H, Guo Y-L, et al. Amelioration of Cavernosal Fibrosis and Erectile Function by Lysyl Oxidase Inhibition in a Rat Model of Cavernous Nerve Injury. J Sex Med 2018;15:304–313.**

Key Words: Cavernous Nerve Injury; Erectile Dysfunction; Lysyl Oxidase; Fibrosis; Rat

INTRODUCTION

Despite technologic advances in radical prostatectomy (RP), such as robotic-assisted nerve-sparing RP, erectile dysfunction (ED) remains a common complication of this surgery.[1,2]

Decreased or lack of nitric oxide release from cavernous nerve (CN) injury (CNI) causes a continuous state of constriction of corpus cavernosum (CC) smooth muscle.[3] The hypoxic state that

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follows can cause serial pathophysiologic alterations in cavernosal tissue, including loss of smooth muscle cells (SMCs) and fibrosis.[4–7] Imbalance between pro-fibrotic and antifibrotic substances has been shown to induce fibrogenesis in the CC.[7–9] Pro-fibrotic substances, such as hypoxia inducible factor-1, transforming growth factor-β (TGF-β), fibronectin (FN), and tissue inhibitors of metalloproteinases (TIMPs), and antifibrotic substances such as matrix metalloproteinases (MMPs), which are a class of molecules responsible for extracellular matrix (ECM) degradation, are found to be aberrantly expressed.[7,8] Fibrous-muscular structural changes in the end organ lead to a difficult-to-treat combination of veno-occlusive dysfunction and neurogenic ED. Preserving the hemodynamic mechanisms of penile erection through the inhibition of fibrosis and SMC loss in the CC could be a promising approach to prevent ED after CNI.[10]

The role of the fibrogenic enzyme lysyl oxidase (LOX) has been demonstrated in the pathogenesis of tissue fibrosis, such as lung fibrosis,[11,12] liver fibrosis,[13] myocardial fibrosis,[14] and skin fibrosis.[15] Among the cytokines that enhance the expression and activity of LOX, the most well established are hypoxia inducible factor-1 and TGF-β1.[16–18] LOX plays a pivotal role in stable and insoluble ECM formation, because of its capacity to convert lysine and hydroxylysine residues in collagen and/or elastin into highly reactive aldehydes, which catalyze the covalent cross-linking of collagen and/or elastin fibers and eventually stabilize fibrous deposits.[14,16,17] In addition, LOX has biological functions that extend beyond this fundamental role, with contributions to angiogenesis, motility and migration in fibroblasts, cell proliferation, cell differentiation, cell signaling, and transcriptional gene regulation.[14,16,18]

In this context, we hypothesized that the hypoxic state after CNI would lead to excessive activation of LOX and that abnormal LOX expression might contribute to the fibrosis of penile tissue. We used a rat model of bilateral CNI (BCNI) to investigate LOX over-activation after CNI and the effects of the specific LOX inhibitor β-aminopropionitrile (BAPN) on erectile function and penile fibrosis.

METHODS

Animals and Treatment

75 male Sprague-Dawley rats (12 weeks old) were randomly divided into 3 equal groups (n = 25 per group): sham operation control (exposure of CN); intraperitoneal injection of phosphate buffered saline vehicle after BCNI; and intraperitoneal injection of BAPN (Sigma-Aldrich, St Louis, MO, USA) dissolved in phosphate buffered saline at a dose of 100 mg/kg once daily after BCNI. This dose was selected based on the published work of others.[13,19] The major pelvic ganglion and CN were exposed after inhalant anesthesia. The sham control rats underwent laparotomy alone. BCNI was performed with a hemostat clamp 5 mm from the major pelvic ganglion on both sides for 2 minutes. Penile tissues were collected after completing erectile functional testing at 30 days after surgery. Experimental protocols complied with the National Institutes of Health Guidelines for the Care and Use of Experimental Animals.

Measurement of Erectile Responses

Intracavernous pressure (ICP) and mean arterial blood pressure (MAP) were measured as previously described.[20,21] Briefly, a 25-gauge needle was inserted into the carotid artery and a 23-gauge needle was inserted into the right crura to monitor MAP and ICP, respectively. Electrostimulation (2.5, 5, 7.5 V, 15 Hz, pulse width = 0.5 ms, duration = 50 seconds) of the CN was applied with a platinum bipolar hook electrode placed proximal to the injury. 3 stimulations were conducted per side. Pressure curves were collected by a data-acquisition system (PowerLab, ADInstruments, Bella Vista, NSW, Australia). The ratio of maximal ICP to MAP and total ICP, which was determined by the area under the curve (millimeters of mercury per second), were calculated.

After measurement, the middle region of the skin-denuded penile shaft was fixed in 10% formalin and then embedded in paraffin for histologic studies. The remaining tissue was harvested immediately for enzyme activity assay or frozen and stored at −80°C.

Histology

Adjacent sections (5 μm) were cut in a transverse direction. The sections were used for Masson trichrome staining for smooth muscle (red) and collagen (blue). For immunohistochemical examination, the sections were incubated with rabbit anti-calponin antibody (1:500; Abcam, Cambridge, MA, USA) and rabbit anti-LOX antibody (1:100; Abcam) overnight at 4°C. The sections were washed and then incubated with the horseradish peroxidase—conjugated secondary antibody. Antigen-antibody reactions were performed with 0.05% diaminobenzidine. At least 3 matched sections per specimen and at least 3 fields in each section were examined. Semiquantitative analysis was carried out using Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA).

Real-Time Quantitative Polymerase Chain Reaction

Total RNA of cavernous tissue was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). As described in our previous experiment,[20] cDNA was synthesized using the PrimeScript RT reagent kit (TaKaRa, Dalian, China) and real-time polymerase chain reaction (PCR) was carried out using SYBR Premix Ex Taq (TaKaRa) with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Los Angeles, CA, USA). LOX, α-smooth muscle actin (α-SMA), MMP2, MMP3, MMP9, TIMP1, TIMP2, COL I, COL IV, and FN mRNA were measured by quantitative PCR using the primers listed in Table 1. PCR was performed in at least 3 replicates. The $2^{-\Delta\Delta Ct}$ method was used for quantification (fold difference) of the expression level.[22] β-Actin was measured as an endogenous reference gene.