Restoration of Erectile Function by Suppression of Corporal Apoptosis, Fibrosis and Corporal Veno-Occlusive Dysfunction with Rho-Kinase Inhibitors in a Rat Model of Cavernous Nerve Injury

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Abbreviations and Acronyms

α-SMA = α-SM actin
CN = cavernous nerve
CNI = CN injury
CVOD = corporal veno-occlusive dysfunction
CVOF = corporal veno-occlusive function
DIC = dynamic infusion cavernosometry
ED = erectile dysfunction
F = I treated with fasudil
I = bilateral CN crush injury
ICP = intracavernous pressure
LIMK = LIM-kinase
MAP = mean arterial pressure
MYPT1 = myosin phosphatase target subunit 1
p = phosphorylated
ROCK1 = RhoA/Rho-kinase
RP = radical prostatectomy
S = sham surgery
SM = smooth muscle
TGF-β = transforming growth factor-β

Purpose: We determined whether Rho-kinase inhibition would improve corporal veno-occlusive dysfunction by suppressing apoptosis and fibrosis via normalization of the Rho-kinase driven pathways related to the 2 structural alterations in a rat model of cavernous nerve crush injury.

Materials and Methods: A total of 30 male 10-week-old male Sprague Dawley® rats were equally divided into 3 groups, including sham surgery, cavernous nerve crush injury and cavernous nerve crush injury treated with fasudil. The treated group received fasudil (30 mg/kg) daily for 4 weeks starting day 1 postoperatively. Electrostimulation and dynamic infusion cavernosometry were performed 4 weeks postoperatively. Penile tissue was processed for imm unohistochemistry, double immunofluorescent and Masson trichrome staining, TUNEL, caspase-3 activity assay and Western blot.

Results: The cavernous nerve crush injury group showed significantly lower intracavernous pressure/mean arterial pressure, and higher maintenance and drop rates than the sham surgery group. Rho-kinase inhibition in the injury plus fasudil group restored erectile responses and dynamic infusion cavernosometry parameters. Increased apoptosis, decreased immunohistochemical staining of α-SMA and increased caspase-3 activity were noted in the injury group. In that group densitometry revealed increased ROCK1 expression, increased MYPT1 phosphorylation, decreased Akt phosphorylation, decreased Bad phosphorylation and a decreased Bcl2-to-Bax ratio. A significantly decreased smooth muscle-to-collagen ratio and increased fibroblast pCofilin were also observed in the injury group, as was increased phosphorylation of cofilin, a downstream effector of LIMK2. Rho-kinase inhibition in the injury plus fasudil group alleviated the histological and molecular dysregulation.

Conclusions: Our data suggest that early inhibition of Rho-kinase after cavernous nerve crush injury may prevent corporal apoptosis and fibrosis by suppressing the Akt/Bad/Bax/caspase-3 and LIMK2/cofilin pathways, preventing corporal veno-occlusive dysfunction and erectile dysfunction.

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DESPITE innovative technical and technological advances during decades the CNs remain exposed and at risk for involuntary damage during nerve sparing radical prostatectomy, leading to ED in men.[1] Corporal apoptosis and fibrosis occur in response to CNI.[2] These changes can cause impaired CVOF, resulting in venous leak and subsequent CVOD.[2] Post-RP ED is primarily attributable to CVOD, which develops progressively after surgery.[2–7] Thus, the important pathophysiology of CVOD and ED after RP includes postoperative corporal apoptosis and fibrosis.

To date various strategies for penile rehabilitation, such as chronic dosing with phosphodiesterase type 5 inhibitors, have been suggested to prevent the functional and structural alterations in the penis induced by CNI during RP.[5] However, there is no conclusive data on efficacy or routine use. Furthermore, the efficacy of phosphodiesterase type 5 inhibitors in men with post-RP ED is lower than in the general population with ED. Although a few studies show that corporal apoptosis and fibrosis can contribute to CVOD after CNI,[3,8,9] little is known about the molecular mechanisms related to the 2 structural alterations that result in CVOD.

We previously reported ultrastructural alterations such as dark shrunken cells in cavernous smooth muscle using electron microscopy in a neurotomized dog model.[10] We recently found that the ROCK1/LIMK2/cofilin pathway could be involved in corporal fibrosis with loss of SM through coordination with TGF-β/sphingosine-1-phosphate signaling after CNI.[11,12] Also, Rho-kinase signaling is involved in apoptosis via modulation of an Akt driven pathway in cardiovascular disease.[13]

Thus, we hypothesized that inhibition of Rho-kinase, a common factor in the TGF-β/Rho-kinase/LIMK mediated pathway and the Rho-kinase/Akt driven pathway, could restore erectile function by suppressing corporal apoptosis and fibrosis, thus, preserving CVOF. We examined whether daily administration of Rho-kinase inhibitors would improve erectile function by suppressing CVOD via anti-apoptotic and antifibrotic effects to better understand the roles of the 2 pathways in the pathophysiological process of post-RP ED.

MATERIALS AND METHODS

Experimental Design

A total of 30 male 10-week-old Sprague Dawley rats weighing 300 to 350 gm were equally divided into 3 groups of 10 each, including S, I and F groups. The F group was treated with once daily oral administration of fasudil (30 mg/kg) for 4 weeks starting from the day after CNI.[14] The S and I groups were treated with oral administration of saline vehicle only.

Rats were anesthetized by intraperitoneal injection of Zoletil® (10 mg/kg) and isoflurane inhalation. In the I group, which approximated the clinical situation in patients undergoing nerve sparing radical prostatectomy, CNI was induced by applying a microsurgical vascular clamp to the CN 2 to 3 mm distal to the major pelvic ganglion for 60 seconds, removing it for 30 seconds and reapplying it for another 60 seconds. In the S group identical pelvic dissection was performed but the 2 CNs were only exposed with no direct manipulation. All CNIs were performed by the same trained surgeon blinded to the random assignment of the experimental groups.

Experimental procedures were approved by the institutional animal care and use committee of the Clinical Research Institute at our hospital, which is an AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) accredited facility. All animals were cared for in accordance with NRC (National Research Council) guidelines for the care and use of laboratory animals.

Erectile Function Assessment In Vivo

Four weeks postoperatively erectile function was evaluated in anesthetized rats as erectile responses to CN electrical stimulation and DIC as described previously.[3,14,15] To assess erectile responses to CN electrical stimulation we introduced a 24-gauge angiocatheter into the carotid artery to continuously monitor MAP. The corpus cavernosum was cannulated with a 26 gauge needle to permit continuous ICP monitoring. After identifying the CNs a platinum bipolar electrode was placed around the CN distal to the site of nerve injury. CN stimulation parameters were 1.0, 2.5 and 4.0 V at 15 Hz with a 0.4 millisecond square wave duration for 30 seconds. Evaluation parameters included ICP/MAP and AUC corresponding to the electrical stimulation duration.

After a 20-minute rest period we performed DIC. Briefly, the contralateral corpus cavernosum was cannulated with another 26 gauge needle. Baseline ICP was recorded and 0.1 ml papaverine (10 mg/ml) was administered through the infusion cannula into the corpora cavernosum. After 5-minute infusion ICP during tumescence was recorded (ICP after papaverine). Saline was infused through the cannula. The infusion rate started at 0.05 ml per minute and increased by 0.05 ml per minute every 10 seconds until an ICP of 80 mm Hg was achieved and maintained (maintenance rate). After ICP remained steady for 20 seconds the infusion was stopped and the ICP decrease was determined in the subsequent 1 minute (drop rate).