Optimization of Sonic hedgehog delivery to the penis from self-assembling nanofiber hydrogels to preserve penile morphology after cavernous nerve injury

Shawn Choe, Elizabeth Kalmanek, Daniel A. Harrington, Samuel I. Stupp, Kevin T. McVary, Carol A. Podlasek*

Abstract/Introduction

Introduction: Erectile dysfunction (ED) is a significant medical condition, with high impact on patient quality of life. Current treatments are minimally effective in prostatectomy, diabetic and aging patients due to injury to the cavernous nerve (CN); loss of innervation causes extensive smooth muscle (SM) apoptosis, increased collagen and ED. Sonic hedgehog (SHH) is a critical regulator of penile SM. We developed a self-assembling peptide amphiphile (PA) nanofiber hydrogel for extended release of SHH protein to the penis after CN injury, to suppress SM apoptosis. We propose that the marked improvements in penile morphology observed with this technology can be significantly further enhanced with optimization of delivery conditions for SHH PA, which is vital for clinical translation.

Methods: Adult Sprague Dawley rats (n=97) underwent: 1.) CN crush with SHH treatment of the penis by PA for 4 days with two SHH protein concentrations, 2.) Increased duration of SHH treatment after CN injury to 9 days with 2 SHH PA injections, 3.) Simultaneous SHH PA delivery to the penis and CN after CN crush. Sham, CN crush only, and MSA PA treated controls were also performed for each group. Apoptotic index, SM, collagen, and proliferation were quantified.

Results: Apoptosis increased 117% 4 days after CN injury. SHH PA suppressed apoptosis 27%. SM was 48% higher with SHH treatment, and doubling the concentration of SHH resulted in higher SM preservation (76%). Increasing the duration of SHH delivery to 9 days with two SHH PA injections continued to suppress apoptosis 22%, and resulted in 100% more SM. Simultaneous SHH PA delivery to the penis and CN was most effective for SM preservation (127%). Proliferative index was increased 50% at 2 days, 38% at 4 days and 31% at 7 days after CN injury. Proliferation occurred in both SM and endothelium.

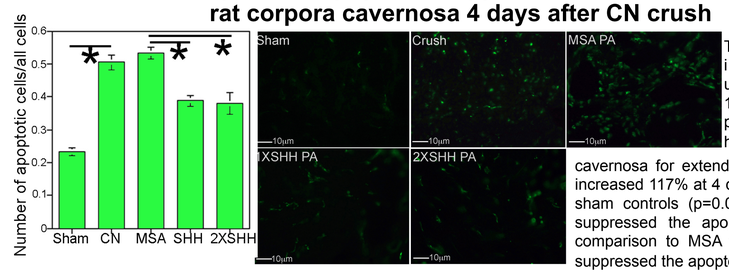
Conclusion: Optimization of sonic hedgehog delivery by PA improved SM preservation to 127%. Dampening the intensity of the early apoptotic response is critical to preserving SM and erectile function. Proliferation of SM and endothelium also occur in the corpora cavernosa after CN injury, and this response is increased with SHH PA treatment. Optimization of sonic hedgehog delivery by PA is indispensable for clinical translation to ED patients to impede erectile dysfunction, and the PA nanofiber distribution mechanism, may be broadly applicable as an *in vivo* delivery tool.

Methods

Animals: Adult Sprague Dawley rats (P115-120, n=97) were obtained from Charles River.
Experimental groups: 1.) Optimization of SHH protein concentration delivered to the penis at the time of CN crush, 2.) Maintain elevated SHH protein longer after CN crush with 2 SHH PA injections or MSA (control), and 3.) Examine additive effects with SHH delivery to both the penis and CN at the time of CN crush. Apoptotic index was evaluated by TUNEL with DAPI counterstain, and smooth muscle and collagen by Image J analysis of trichrome stain.
CN Crush: Bilateral CN crush was performed by exposing the pelvic ganglia (PG) and cavernous nerve and the CN was crushed for 30 seconds using microforceps (0.02x0.06mm). A change in color and indent of the CN was observed. Sham surgery was performed by exposing but not damaging the CN. Rats were sacrificed after 4 (n=12) or 9 (n=10) days.
Concentration dependent apoptosis suppression: CN crush was performed and either SHH (n=5) or MSA (control, n=4) protein was injected via PA hydrogel ($V_3A_3E_3$-COOH) into the corpora cavernosa of the penis (Bond et al., 2011), where the PA formed a loose gel lining the sinusoidal spaces and allowed for extended release of protein. The final amount of SHH protein injected was 6.25 µg per rat (1X) or 2X, delivered by PA (n=5) into Sprague Dawley rats.
Apoptosis suppression with extended release: CN injury and injection were performed with SHH or MSA/BSA PA as described above. At day 5, when SHH protein is largely depleted from the PA as it breaks down, a second SHH (n=8) or MSA/BSA (control, n=7) PA injection was performed and rats were sacrificed after an additional 4 days. A second group that had been given only one SHH PA injection at the time of CN crush was also performed, and rats were sacrificed after 9 days (n=4).
Simultaneous treatment of the CN and penis: CN injury was performed and rats were immediately injected into the corpora cavernosa with SHH (n=7) or MSA (n=4, 1 µg/µl) PA as described above. Following injection into the penis, SHH or MSA was delivered bilaterally to the PG/CN using a second type of PA (1.5 µg per PG/CN and 3 µg per rat, Angeloni et al., 2011).
Trichrome: Trichrome stain was quantified by Image J analysis with background subtraction.
Apoptotic Index: TUNEL assay was performed using Apoptag kit (Millipore) with DAPI counterstain for all cells, on frozen penis tissue as described previously (Angeloni et al., 2011).
Statistics: Statistics were performed using ANOVA and a Scheffe's posthoc test.

Results

1. Concentration dependent apoptosis suppression with SHH protein treatment in rat corpora cavernosa 4 days after CN crush



The apoptotic index was quantified in Sprague Dawley rats that underwent bilateral CN crush and 1X or 2X SHH protein delivered by peptide amphiphile (PA) nanofiber hydrogel injected into the corpora cavernosa for extended protein release. Apoptosis was increased 117% at 4 days after CN injury in comparison to sham controls (p=0.0001). 1X SHH protein treatment suppressed the apoptotic index by 27% (p=0.005) in comparison to MSA treated controls. 2X SHH protein suppressed the apoptotic index by 29% (p=0.003).

2. Concentration dependent smooth muscle preservation with SHH treatment by PA

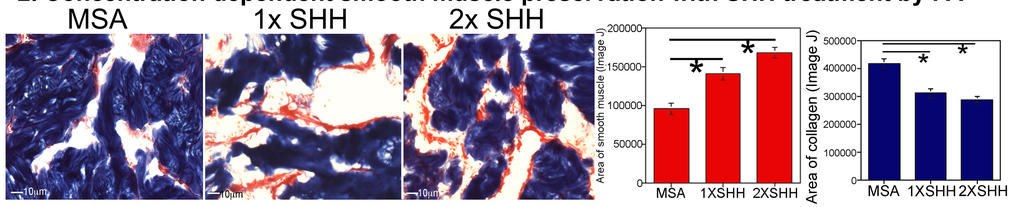
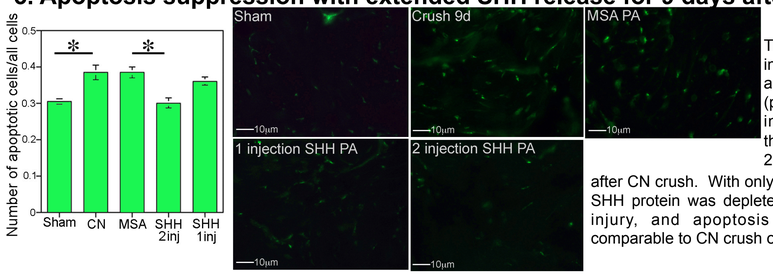


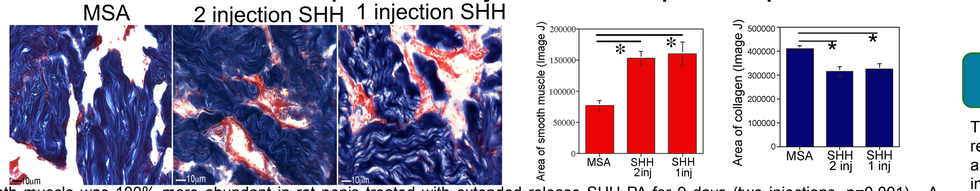
Image J analysis of trichrome stain showed 48% more smooth muscle with 1X SHH treatment (p=0.005) and 76% more smooth muscle with 2X SHH treatment (p=0.0001) in comparison to MSA treated control, and 26% less collagen in the 1X (p=0.002) and 32% collagen in the 2X SHH treated group (p=0.0001).

3. Apoptosis suppression with extended SHH release for 9 days after CN crush by PA



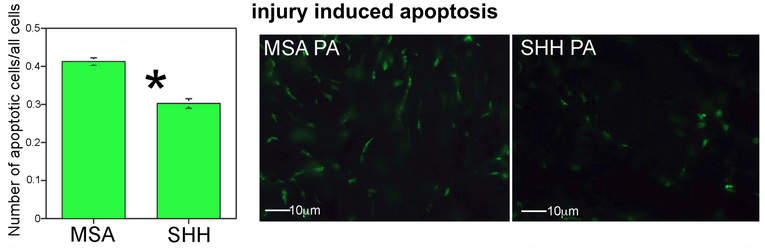
The apoptotic index was increased 26% at 9 days after CN injury (p=0.014). Two SHH PA injections suppressed the apoptotic index by 22% (p=0.021) at 9 days after CN crush. With only one SHH PA injection, SHH protein was depleted by 9 days after CN injury, and apoptosis returned to levels comparable to CN crush only.

4. Extended release of SHH protein for 9 days after CN crush preserves penile SM



Smooth muscle was 100% more abundant in rat penis treated with extended release SHH PA for 9 days (two injections, p=0.001). A comparable 110% increase in penile smooth muscle was identified with one SHH PA injection (p=0.001). Collagen was 24% less in the 2X SHH (p=0.003) and 21% less in the 1X SHH (p=0.022) treated group

5. Simultaneous treatment of the CN and penis with SHH PA suppresses CN injury induced apoptosis

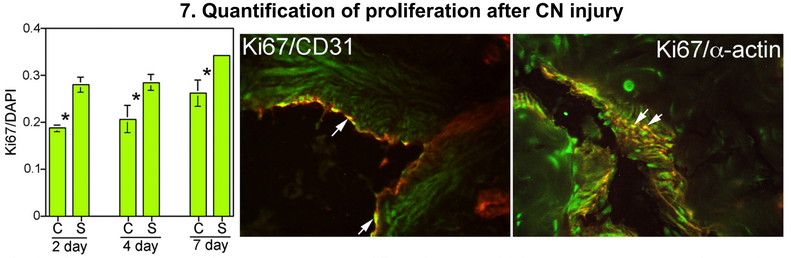


The apoptotic index was examined at 4 days after CN injury in Sprague Dawley rats treated with SHH PA or control (MSA PA) on top of the crushed CN, and at the same time in the corpora cavernosa of the penis. Apoptosis was suppressed 27% at 4 days after CN injury (p=0.0001)

6. Simultaneous treatment of the CN and penis at the time of CN injury preserves smooth muscle

Smooth muscle was 127% (p=0.0004) more abundant at 4 days after CN injury with SHH PA treatment of both the CN and penis at the time of CN injury, and collagen was 30% less abundant (p=0.0003).

7. Quantification of proliferation after CN injury



Proliferative index was quantified by measuring Ki67 positive cells/DAPI in corpora cavernosal tissue of Sprague Dawley rats that underwent CN injury and were treated with SHH or BSA (control) by PA for 2, 4 and 7 days. Proliferation increased 50% at 2 days, 38% at 4 days and 31% at 7 days of SHH treatment. Proliferation occurred in smooth muscle and endothelium.

Conclusions

The level of apoptosis suppression was similar in previous CN resection, and the present CN crush model, in response to SHH treatment by peptide amphiphile (PA) nanofiber hydrogel. One SHH PA injection suppressed apoptosis until SHH protein was depleted from the PA. Maintaining SHH abundance throughout the apoptotic CN injury response, by a second SHH PA injection, maintains suppression of apoptosis longer, and improves penile morphology. Simultaneous delivery of SHH to penis and CN, preserved the most smooth muscle. Optimization of SHH PA delivery is essential for translation to prostatectomy patients to prevent ED.

Source of Funding: National Institutes of Health/National Institute of Diabetes and Digestive and Kidney Diseases DK101536.