Future Targets

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Pathways activating cGMP

- HO-1
- CO
- NO
- CNP
- Natriuretic peptides
- Opiorphin

Processes:
- sGuanylate cyclase
- pGuanylate cyclase
- cGMP
- Relaxation
- Smooth Muscle
cAMP signaling pathways

ATP → cAMP → PKA → Ca\(^{2+}\) → Relaxation

- Alprostadil
- Adenosine
- PGE1R
- A2br
- Adenylate cyclase
Adenosine is generated both intra- and extracellularly.
H₂S involvement in erectile physiology

Endothelial Cell

L-cysteine

Cystathionine γ-lyase (CSE)

Smooth Muscle Cell

L-cysteine

cystathionine β-synthase (CBS) and CSE

cAMP/cGMP?

K-ATP-Channels

Ca²⁺

K⁺
Vasoconstrictive Pathways

- Endothelin-1
- Norepinephrine
- Serotonin
- Angiotensin

**RELAXATION**
- MyosinLC20
- MLCK
- Ca^{2+} - CaM

**CONTRACTION**
- MyosinLC20.P

**PKC**
- PLC
- DAG

**ROCK**
- CPI-17
- RhoA-GTP
Future Targets

• Rho-kinase
• Arginase
• Adenosine
• sGC activators
• Stem Cells/Tissue engineering
Future Targets

• Rho-kinase *
• Arginase
• Adenosine
• sGC activators
• Stem Cells/Tissue engineering *

* Most promising targets at this time.
Activation of RhoA / Rho-Kinase Inhibits Corporal Relaxation

- HTN, DM, Hypoxia, NE, ET-1 stimulate MLC~P contraction
  - MLC (relaxation)
  - MLC-KINASE
  - MLC~P (contraction)

- RHOA~GDP (INACTIVE) binds GTP and migrates to membrane modifications
  - RHOA~GTP (ACTIVE)
  - RHO-KINASE + ATP
  - MLC PHOSPHATASE~P (INACTIVE)
  - PHOSPHATASE PHOSPHATASE (?)
  - eNOS protein
  - eNOS mRNA stability

- Fibrosis/Apoptosis

- MLC PHOSPHATASE (ACTIVE)

- eNOS Protein
  - eNOS mRNA stability

- Activation of RhoA / Rho-Kinase Inhibits Corporal Relaxation
Inhibition of RhoA / Rho-Kinase Enables Corporal Relaxation

- **MLC PHOSPHATASE**
- **MLC PHOSPHATASE~P**
- **RHO-KINASE**
- **RHOA~GDP (INACTIVE)**
- **RHOA~GTP (ACTIVE)**
- **NO/cGMP/PKG INHIBIT**
- **MLC (RELAXATION)**
- **MLC KINASE**
- **MLC~P (CONTRACTION)**
- **ERECTION**
- **eNOS Protein**
- **eNOS mRNA stability**
- **PHOSPHATASE PHOSPHATASE (?)**
- **MLC PHOSPHATASE (ACTIVE)**
- **MLC PHOSPHATASE~P (INACTIVE)**
Effect of Intracavernous Injection of the Rho-kinase Inhibitor, Y-27632
Common Systemic Diseases Associated with ED

- Diabetes: 40%
- Vascular Disease: 30%
- Spinal Cord Injury: 8%
- Radical Surgery: 13%
- Endocrine Disorders: 6%
- Multiple Sclerosis: 3%

Rho-Kinase Inhibition Reduces Apoptosis and Preserves SMC content in Diabetic Rats

WJ Li et al., JSM 2011
Rho-Kinase Inhibition Reduces Intimal Thicking in Internal Iliac Artery

K Park, et al. JSM 2006
Rho-Kinase Inhibition Improves Endothelial NOS and Prevents ED

K Park, et al. JSM 2006
Strategies to promote axon regeneration

1. Stimulation of axon regeneration by modulating the neuronal signaling responses:
   - Treatment with neurotrophic factors (NGF, BDNF, NT-3, GDNF, LIF, FGF-2)

2. Cell transplantation: e.g. embryonic stem cells, adipose-muscle-, bone marrow derived -mesenchymal stem cells.

3. Neutralization of the inhibitory factors in the injured PNS
   - A therapeutic vaccine approach
   - Anti-scarring treatment (inhibition of fibroblast proliferation)
   - Anti-inflammatory / Antioxidants
   - Immune modulators
   - Anti-apoptotic agents
   - *Axonal outgrowth inhibitory neutralizers*
• RhoA mediates growth inhibitor signals which stiffens the actin cytoskeleton, thereby inhibiting axonal elongation.

• RhoA is a key inhibitory regulator of axonal regeneration in the CNS, however, little is known in the PNS.
Bilateral Cavernous Nerve Injury (BCNI)

Major Pelvic Ganglion (MPG)  
Cavernous Nerve Injury with forceps 15 sec x 3  
Major Pelvic Ganglion (MPG)

Left CN
Right CN

http://www.pelvipharm.com
Cellular responses to peripheral nerve injury

- Failed regeneration after delay
- Schwann cells tubes deteriorate: mechanism unknown
- Atrophied target muscle prevents reinnervation

Major Pelvic Ganglion (MPG)

Apoptosis occurs in some neurons after axotomy

Axons are unable to regenerate through unsupportive distal nerve

Penis

Erectile Dysfunction

ICP/MAP

Volts

Sham
BCNI 48h
BCNI 14d
BCNI 7d
BCNI 30d
BCNI 60d
Major Pelvic Ganglion (MPG)

Penis

Inflammatory Cytokines upregulate RhoA/Rho-kinase

↑ Apoptosis (via caspase)

↓ neuronal nitric oxide synthase

Wallerian Degeneration

Schwann cells tubes deteriorate: mechanism unknown

Erectile Dysfunction

Hypothesis
BCNI significantly elevated RhoA/Rho-kinase

![Graphs showing expression levels of RhoA, ROCK1, and ROCK2 over time with BCNI and Sham conditions.](Image)
Effect of ROCK Inhibition on the MPG?

Y-27632 → Rho-kinase (ROCK) inhibitor

5 mg/kg i.p. BID *(dose selective for ROCK isoforms)*

Day 0:
Sham/
BCNI

Day 14:
ICP, tissue
collection

Y-27632
ROCK inhibition preserves cavernous nerve structure

- Increased myelinated axons
- Strong presence of Schwann cells
Hannan JL et al., Molecular Neuroscience 2014
Inhibition of ROCK: Effect on MPG Neuritogenesis

48h incubation

Sham

Y27632

Neurite Length (μm)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Neurite Length (μm)</th>
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<tbody>
<tr>
<td>Sham</td>
<td></td>
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<tr>
<td>BCNI14d</td>
<td></td>
</tr>
<tr>
<td>BCNI14d+Y27632</td>
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**Note:** The graph shows a comparison of neurite length among different samples. The bars indicate a significant difference (**p < 0.01**) between the Sham and BCNI14d+Y27632 groups, suggesting that Y27632, an inhibitor of ROCK, enhances neuritogenesis compared to Sham and BCNI14d conditions.
Improved nerve-mediated responses after ROCK inhibition

* p<0.05 vs Sham; Ψ p<0.05 vs Sham and BCNI; δ P<0.05 vs BCNI
Figure 3

(a) nNOS/GAPDH

(b) m-eNOS/GAPDH

J Hannan et al, J Urol 2012
**Figure 1**

- **a:**
  - Voltage vs. Peak ICP (mmHg)
  - Bars represent different treatment groups: SHAM (n=8), CNI (n=8), and CNI + Y-27632 (n=7).
  - Significant differences indicated by asterisks.

- **b:**
  - Voltage vs. ICP/MAP
  - Significant differences indicated by asterisks.

- **c:**
  - Voltage vs. Total ICP (Area Under the Curve; mmHg*s)
  - Significant differences indicated by asterisks.

- **d:**
  - Voltage vs. T80 (s)
  - Significant differences indicated by asterisks.

*J Hannan et al, J Urol 2012*
Stem cells

**Immunomodulation**
- iDC
- mDC
- PGE-2
- HLA-G5
- HGF, iNOS
- PGE-2
- TGFβ1
- IDO
- H2AX
- Tryp-L-kynurenine
- picolinic acid

**Anti-apoptosis**
- VEGF
- HGF
- IGF-1
- Sema6D
- TGFβ
- bFGF
- GM-CSF
- Trp-5-L-kynurenine
- picolinic acid

**Angiogenesis**
- VEGF
- IGF-1
- PI GF
- MCP-1
- IL-6
- ECM molecules

**Support of growth and differentiation of stem and progenitor cells**
- SCF
- LIF
- M-CSF
- SDF-1
- Angiopoietin-1

**Anti-scarring (anti-fibrosis)**
- HGF
- bFGF
- Adrenomedullin (?)

**Chemoattraction**
- CCL2
- CCL3
- CCL4
- CCL5
- CCL6
- CCL20
- CCL26
- CX3CL1
- CXCL5
- CXCL11
- CXCL1
- CXCL2
- CXCL8
- CXCL10
- CXCL12
STEM CELL TREATMENT FOR ERECTILE DYSFUNCTION

- Clinical need
- Types of stem cells
- Efficacy and mechanisms of action
  - ED
    - cavernous nerve injury
    - (aging)
    - (diabetes and metabolic syndrome)
  - Peyronie’s disease
Medline Search – Stem Cells and Erectile Dysfunction

Total - 136
Stem Cells and Erectile Dysfunction

- Animal Models used to study stem cell-based therapies.
  - Aging
  - Hypogonadism
  - Hypercholesterolemia, Arterial insufficiency.
  - Diabetes mellitus type 1 and 2 (Metabolic Syndrome)
  - Cavernous nerve injury and resection.
Stem Cell Biology

- Biopsy
- Aspirate

Skeletal Muscle

Muscle Cells

Digestion

Preplate Procedure

Bone Marrow

Liposuction

Centrifugation

BM-MNC

BMSC

Adipose Tissue

Digestion and Centrifugation

SVF

Plating

ADSC
Stem cell research in ED

LacZ (gene)
GFP (gene)
BrdU (DNA-incorporation)
EdU (DNA-incorporation)
CM-Dil (cytoplasm)
PKH26 (cell membrane)

Ex-Vivo Gene Modifications (eNOS, VEGF)
Stem cells

1) diseased/lost cell replacement
2) paracrine modification of natural coarse of disease or paracrine stimulation of trophic processes
Dual effects: neural tissues vs penile tissues

Primary:

CAVERNOUS NERVE INJURY

Axonotmesis

\[ \text{TNF-\(\alpha\)} \]
\[ \text{TGF-\(\beta_1\)} \]

\[ \text{chemokines} \]

\[ \text{neuro-inflammation} \]

\[ \text{Wallerian degeneration} \]

\[ \downarrow \text{NO bioavailability} \]
Dual effects:
neural tissues vs *penile tissues*

Secondary:

CAVERNOSAL DENERVATION

- hypoxia
  - TGF-β,
  - smooth muscle apoptosis
  - collagen deposition (fibrosis)
  - veno-occlusive dysfunction

Images:
- Sham
- BCNI 14d
- BCNI 30d
  - 2x
  - 10x
adipose tissue-derived stem cells (ADSC)

bone marrow derived stem cells (BMDSC)
ADSC recovers erectile function in cavernous nerve injured rats

**Figure 1** Left panel: representative recordings of intracavernous pressure (ICP)-registration upon stimulation of the distal cavernous nerve (CN). Black bar represents one electrical stimulus of 50 seconds. Right panel: results of ICP-measurement expressed as the ratio ICP/ mean arterial pressure (MAP). *P < 0.05 compared with vehicle-treated group. ADSC = adipose tissue-derived stem cell.

J Sex Med 2010;7:3331–3340
Preserved nNOS expression in dorsal penile nerve

Figure 2 Left panel: representative images of the dorsal penile nerve of each group. Original magnification ×400. Right panel: result of nitric oxide synthase (nNOS) quantification expressed as the number of nNOS-expressing fibers/area of the nerve (in pixel). *P < 0.05 compared with vehicle-treated group. ADSC = adipose tissue-derived stem cell.

J Sex Med 2010;7:3331–3340
Proteomic profile angiogenesis array analysis of human ADSCs

Guihua Liu et al, PLOS One 8: 2013
Mesenchymal Stem Cells (ADSC) demonstrate neurotrophic effects on cavernous nerve regeneration.
Nerve Grafts to replace CN

Biomaterials with Schwann cells, Growth factors, Rho-kinase inhibitors, Neurotrophic factors, stem cells

Hannan JL, Mao M, Bivalacqua TJ
Stem Cell Based Clinical Trials


- Clinical trial in France using IC MSCs – ongoing.

- Industry sponsored IC ADSCs for post-RP ED.

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<th>With sildenafil citrate</th>
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5/7 clinical response
Stem Cell Based ED Clinical Trial at Johns Hopkins: PRIMES 2014

- PRIMES (PREventing Impotence with MEsenchymal Stem Cells) trial for post-radical prostatectomy ED.
- PI: Medical Oncology – Sam Denmeade M.D. Ph.D. and Johns Isaacs Ph.D
- PI: Urology – Trinity J. Bivalacqua M.D. Ph.D Alan Partwin M.D. Ph.D.
- Protocol – Intravenous injection of MSCs prior to RP to prevent degeneration of cavernous nerves and identification in prostate cancer.
Conclusion

• Development of pharmacological agents targeting a number of molecular pathways are necessary for treatment of PDE5 inhibitor non-responders.
  – However, is there interest to invest in their development for treatment of ED.

• Stem cell based therapies have support in pre-clinical animal models and clinical trials results are forthcoming to determine efficacy in men with ED.
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