The Molecular Mechanisms of Cavernous Nerve Response to Injury at Prostate Cancer Surgery: Identifying Translational Targets to Impact Patient Care

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ACKNOWLEDGEMENTS

Research Funding
Prostate Cancer Research Foundation of Canada
Canadian Male Sexual Health Council
Canadian Foundation for Innovation Infrastructure Grant
Northeastern Section of the American Urological Association
Acorda Therapeutics

Greta and John Hansen Chair in Men’s Health Research
OUTLINE

• Identify the translational need for optimizing recovery of cavernous nerve injury

• Review nerve cell and neurovascular bundle function in this clinical context
OUTLINE

• Identify the translational need for optimizing recovery of cavernous nerve injury

• Review nerve cell and neurovascular bundle function in this clinical context

• Outline select research programs with translational potential for this patient group

INTRODUCTION

• Prostate cancer is the leading solid-organ cancer among men in the United States and the second leading cause of cancer deaths

• Radical prostatectomy is a major option for treatment of clinically localized prostate cancers

• Operative morbidity can result in a significant decrease in patient quality of life, even if surgery itself is curative
"Treatability" of Prostate Cancer
2012 = Long Term Survival

Importance of ED Treatment

- After fear of death/disability and incontinence, ED is the most common concern for the patient after prostate cancer treatment

- Why?
  - Acute nature of the ED
  - Last step to normalcy
Defining the Impact

- Critical review of contemporary literature would suggest that regardless of surgical approach, the probability of clinically compromised potency is greater than 2 in 3

IS PRESERVATION OF ERECTILE FUNCTION A PATIENT PRIORITY?

- After fear of death and incontinence, ED is the most common concern for the patient after prostate cancer treatment
  - Impotence
  - Poor self-esteem
  - Depression
  - Difficulty with interpersonal relationships
  - Decreased quality of life
NOT ONLY THE PROSTATE CANCER POPULATION

- Laparoscopic Pelvic Autonomic Nerve-Preserving Surgery for Patients with Lower Rectal Cancer after Chemoradiation Therapy
- Impact of laparoscopic surgery on bladder and sexual function after total mesorectal excision for rectal cancer
- Male Urinary Function After Combined Nerve-Sparing Total Mesorectal Excision with Dissection in Front of Denovilliers’ Fascia

ETIOLOGY OF ERECTILE DYSFUNCTION

- Arterial
  - Arterial
  - Arteriolar
- Cavernosal
  - Tunica albuginea
  - Cavernous muscle
  - Endothelium
  - Fibroblastic Trabeculae
  - Emissary Vein
- Systemic Diseases
- Psychological
- Drugs
- Neurological
  - Sensory
  - Motor
  - Autonomic
  - Neurotransmitters
- Hormonal
  - Testicular
  - Pituitary
  - Thyroid
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Cavernous Nerve

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Basic Penile Physiology

• Cavernous nerves parasympathetic branches (S2-4) are excitatory, releasing nitric oxide

• NO increases cyclic guanosine monophosphatase levels (via soluble guanylyl cyclase)

• Smooth muscle relaxation occurs (protein kinase G), arterial inflow increases

NERVE CELL BASIC SCIENCE

• Synthesis of nitric oxide and subsequent binding to soluble guanylyl cyclase, facilitating GTP to cGMP conversion

• NO liberated immediately by nNOS from substrate L-arginine

• SM cell relaxation as calcium levels decrease
Cavernous Nerve Anatomic Distribution

Why Does ED Occur?

- Neurogenic (nerves are the only 'on' signal)
  - Neural trauma leads structural changes in erectile tissue
EMBRYOLOGY, ANATOMY AND PERIPHERAL NERVE INJURY

- Inferior hypogastric plexus - lamellate arrangement of ganglia, multiplicity of sympathetic and parasympathetic roots, and complexity of terminal branches

- 3-dimensional cartography of neurotransmitter distribution

Coexistence of adrenergic and cholinergic nerves in the inferior hypogastric plexus: anatomical and immunohistochemical study with 3D reconstruction in human male fetus

Bayan Aalad,1,3 Thomas Besiede,1 Ibrahim Karam,1,2 Isam Abd-Alaamad,1 Jean-François Lih,1 Gérald Benoit,1 Stéphanie Drouzy,1 and Vincent Delmas1

1Laboratory of Experimental Surgery, EA-4122, Faculty of Medicine, Bicêtre-Paris 11 University, Le Kremlin-Bicêtre, France

Institute of Anatomy of Female Nerves, Faculty of Medicine, René Descartes/Paris 5 University, Paris, France
• 16 week old fetus

• Fibers of CN destined for cavernosa
• 16 week old fetus
• Fibers of CN destined for cavernosa
• Immunohistochemical staining for cholinergic (VChT) and adrenergic (TH) fibers
• Green adrenergic
• Purple cholinergic

• In "C" - cholinergic branches continue to corpora
• Confirms dorsal and cavernous n. communication
• Cavernous spray-like pattern
HOW WELL DO WE SPARE NERVES DURING RADICAL PROSTATECTOMY?

Nerve sparing = Sheath intact

- Schwann cells
- Basal lamina (laminin)
- Permissive growth environment

Growth Cone of axon
Non nerve-sparing

Nerve sheath disrupted and interposed by scar tissue

NERVE DISTRIBUTION ALONG THE PROSTATIC CAPSULE

- Permanent sections of 31 pts (non-nerve-sparing RP)
- Whole mounts
- Analysis of the distribution of the nerves along the prostatic capsule

Eichelberg et al Eur Urol 2007;51:105-111
NERVE DISTRIBUTION ALONG THE PROSTATIC CAPSULE

20-25% of nerve fibers in the ventral circumference

Eichelberg et al, Eur Urol 2007;51:105-111

Topographical Anatomy of Periprostatic and Capsular Nerves: Quantification and Computerised Planimetry

Roman Ganzer*, Andreas Blana®, Andreas Gaumann®, Jens-Uwe Stolzenburg®, Robert Rabenalt®, Thorsten Bach®, Wolf F. Wieland®, Stefan Denzinger®

- Thirty whole-mount sections
- Non-nerve-sparing radical prostatectomy
- Immunohistochemical nerve staining
- Variability between specimens

Ganzer et al, Eur Urol 2009
Topographical Anatomy of Periprostatic and Capsular Nerves: Quantification and Computerised Planimetry

Roman Ganzer a,b, Andreas Blana a, Andreas Gaumann b, Jens-Uwe Stolzenburg c, Robert Rabenalt d, Thorsten Bach e, Wolf P. Wieland f, Stefan Denzinger a

Capsular nerves
Topographical Anatomy of Periprostatic and Capsular Nerves: Quantification and Computerised Planimetry

Roman Ganzer¹, Andreas Blana³, AndreasGaumann⁴, Jens-Uwe Stolzenburg⁵, Robert Rabemalt⁶, Thorsten Bach⁷, Wolf F. Wieland⁸, Stefan Denzinger⁹

Ganzer et al, Eur Urol 2009

The Periprostatic Autonomic Nerves—Bundle or Layer?

Karl-Dietrich Sievert¹, Jörg Hennenlotter¹, Ines Laible, Bastian Amend, David Schilling, Aristotelis Anastasiadis, Ursula Kuehs, Udo Nagele, Arnulf Stenzl

Department of Urology, University of Tuebingen, Tuebingen, Germany

Sievert et al, Eur Urol 2009
The circumference of the prostate capsule was electrically stimulated during RRP in 12 pts (localized PCa)
• The circumference of the prostate capsule was electrically stimulated during RRP in 12 pts (localized PCa)

• Periprostatic nerve fibers at the 12 through 5 o’clock positions of the midprostate were stimulated using bipolar electrodes

Kaiho et al, Eur Urol 2009
Mean amplitude of pressure responses was most powerful at the 5 o’clock position.

Kaiho et al, Eur Urol 2009

CLINICAL IMPLICATIONS?
SURGICAL APPROACH TO NERVE-SPARING PROCEDURE
INTERFASCIAL VS. INTRAFASCIAL

Standard (interfascial) nerve-sparing prostatectomy

Intrafascial nerve-sparing prostatectomy

CLINICAL IMPLICATION:
SURGERY AS A NEURAL STRATEGY
2D and 3D T2-weighted MR sequences for the assessment of neurovascular bundle changes after nerve-sparing radical retropubic prostatectomy with erectile function correlation

• Cavernous nerve with between fiber edema

Nerve Growth Factor Modulation of the Cavernous Nerve Response to Injury

Anthony J. Bella, MD,‡,‡ Guiling Lin, MD, PhD,‡ Ching-Shwun Lin, PhD,‡,‡ Duane R. Hickling, MD,‡ Christopher Morash, MD, and Tom F. Lue, MD‡ J Sex Med 2009;6(suppl 3):347–352

Sonic Hedgehog, Apoptosis, and the Penis

Carol A. Podlasak, PhD J Sex Med 2009;6(suppl 3):334–335

Role of Immunophilins in Recovery of Erectile Function after Cavernous Nerve Injury

Sena F. Sezen, PhD, Gwen Lagoda, MS, and Arthur L. Burnett, MD J Sex Med 2009;6(suppl 3):340–346

A New Strategy, SuperEnzyme Gene Therapy in Penile Rehabilitation

Jiuhong Yuan, MD,* O. Lenaine Westney, MD,* Ke-He Ruan, MD, PhD,† and Run Wang, MD*
ETIOLOGY OF ERECTILE DYSFUNCTION

**Psychological**
- Systemic Diseases
- Drugs

**Neurological**
- Sensory
- Motor
- Autonomic
- Neurotransmitters

**Hormonal**
- Testicular
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**Cavernous Nerve**
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IS THE NERVE CELL CLINICALLY RELEVANT?

MULTI-ORGAN SYSTEM FAILURE
IS THE NERVE CELL CLINICALLY RELEVANT?

MULTI-SYSTEM ORGAN FAILURE

PATHOGENESIS AND TREATMENT

Primary insult (surgery) → Secondary effects
Injury to cavernous nerve (+/- accessory pudendal artery) → Apoptosis, Hypoxia, Fibrosis, Smooth muscle loss

Injections
Oral agents

???
"SITTING DUCK TARGET?"

'CLASSIC' NEUROTROPHIC FACTORS

- Neurotrophins have essential roles in the survival, development and differentiation of neurons in the central and peripheral nervous systems.
ENDOGENOUS RESPONSE AFTER INJURY

• Collateral sprouting of axons occurs acutely following injury to adult peripheral neurons

• Growth cones target local environments supportive of regeneration

• Molecular mechanisms of this process remain incompletely understood for parasympathetic neurons

'CLASSIC' NEUROTROPHIC FACTORS

• Functional recovery post-injury remains slow and incomplete

• Assumption: limitation in nerve regeneration or morphological penile changes secondary to loss of innervation
CAVERNOUS NERVE REGENERATION: ENDOGENOUS

• Nitrergic axon regeneration following bilateral penile nerve crush

• nNOS immunoreactive fibers corpus cavernosum from intact, and acutely (3–4 week) and chronically (10–12 week) injured rats

• Scale bar=100 μm

CAVERNOUS NERVE REGENERATION: ENDOGENOUS

- Nitrergic axon regeneration following bilateral penile nerve crush
- nNOS immunoreactive fibers corpus cavernosum from intact, and acutely (3–4 week) and chronically (10–12 week) injured rats
- Scale bar=100 μm

• Frequency–response curves for nerve-evoked relaxations from intact, acute (3–4 week) and chronic (10–12) injured rats

'CLASSIC' NEUROTROPHIC FACTORS: RATIONALE

- Regeneration of penile nitrergic axons has been demonstrated
- Function is compromised
- Mechanisms: presynaptic NO synthesis or release (even as regenerated axons express NOS), target-tissue changes, or combination

'CLASSIC' NEUROTROPHIC FACTORS

- Nerve growth factor (NGF) and brain derived neurotrophic factor (BDNF) investigated as peripheral nerve neuromodulators
- Members of the neurotrophin (NT) family which include neurotrophin NT 3 and NT 4/5
'CLASSIC' NEUROTROPHIC FACTORS

- NTs bind to two distinct classes of glycosylated receptor: the p75 neurotrophin receptor -p75NTR- and tyrosine kinase receptors (TRKs)

- Whereas p75NTR binds to all NTs, the TRK subtypes are specific for each NT

DECREASED IN-VIVO BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) EXPRESSION IN AGED RATS FOLLOWING CAVERNOUS NERVE AXOTOMY: A MOLECULAR TARGET FOR ENHANCING NERVE RECOVERY

Anthony J. Bella¹, Guiting Lin², Lia Banie², Kavirach Tantiwongse², William O. Brant³ and Tom F. Lue²

University of Ottawa, Canada
University of California, San Francisco
University of Colorado, Denver
BDNF- ENHANCED GROWTH

- JAK/STAT is the primary molecular signaling pathway for \textit{in vitro} BDNF-induced cavernous nerve regeneration

BDNF- ENHANCED GROWTH

- Western-blot identification of phosphorylation states

IN-VIVO BDNF RESPONSE FOLLOWING TRANSECTION

Bella et al, Eur Urol 52(2); 574-81, 2007
IDENTIFICATION OF TrkB, TrkC, AND P75 NEUROTROPHIN RECEPTORS

TrkB and TrkC

Brain-Derived Neurotrophic Factor (BDNF) Acts Primarily via the JAK/STAT Pathway to Promote Neurite Growth in the Major Pelvic Ganglion of the Rat: Part 2

Guling Lin, MD, PhD, Anthony J. Belka, MD, Tim F. Lu, MD, and Ching-Shayan Lu, PhD
**BDNF- ENHANCED GROWTH**

DCR-MPG Immunohistochemistry
(A) positive S-100 staining indicative of Schwann cells
(B) negative control.

BDNF-induced cytokine secretion from Schwann cells:
MCP-1, MCP-3, oncostatin M, TNF-a, and SDF-1

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**BDNF, AGING AND NEUROBIOLOGY**

- Patient age is amongst the most powerful predictors of post-operative erectile dysfunction after treatment for prostate cancer
- Growth factors aid in neuroregenerative and neuroprotective roles for the cavernous nerves
- The effect of aging on the BDNF response in vivo has not been previously described
EXOGENOUS BDNF EFFECTS ON NEURITE OUTGROWTH IN AGED RATS

- Tissue culture: differential effects of BDNF on the major pelvic ganglion and cavernous nerve when young and aged rats were compared

ENDOGENOUS BDNF RESPONSE TO CAVERNOUS NERVE INJURY IN AGED RATS

- Male Fisher 344 rats underwent cavernous nerve transection and subsequent harvest of the MPG and cavernous nerve segment

- BDNF expression in the penis and MPG were quantified using RT-PCR. Western blot was used for confirmation

- Immunohistochemical analyses identified receptors specific for BDNF and the JAK/STAT pathway in the MPG
ENDOGENOUS BDNF RESPONSE TO CAVERNOUS NERVE INJURY IN AGED RATS

BDNF expression in MPG and penis after CN transection
(Normalized by control)

Young

Old

MPG

Penis

ENDOGENOUS BDNF RESPONSE TO CAVERNOUS NERVE INJURY IN AGED RATS

BDNF expression in MPG and penis after CN transection
(Normalized by control)

Young

Old

MPG

Penis
The JAK/STAT pathway was activated in the MPG following transection but decrease in the aged group (<0.05)
ENDOGENOUS BDNF RESPONSE TO CAVERNOUS NERVE INJURY IN AGED RATS

- BDNF expression in the end-organ target for neuroregeneration, the penis, was significantly decreased in aged animals.

- JAK/STAT pathway activation, the primary molecular signaling mechanism for BDNF enhanced neurite growth was decreased in the MPG of older animals.

Together, these findings suggest that the growth factor response is blunted as part of the aging process.

Strategies to enhance BDNF and other growth factor responses to cavernous nerve injury may translate into increased functional preservation of the nerve, and consequently, erectile function.
DOES THE BDNF STORY MATTER?

"..importantly provide new insight with respect to mechanisms of cavernous nerve functional recovery in the face of pelvic trauma or disease states with relevance to erectile function preservation...[and] elucidation of the molecular mechanism of action for BDNF may be transferable to actions of other neuromodulatory therapies under investigation"

(Dr Arthur Burnett, Johns Hopkins)

GLIAL GROWTH FACTOR 2
Remote from injury-site delivery of glial growth factor 2 (GGF2) facilitates recovery of erectile dysfunction following bilateral cavernous nerve injury in the rat with direct evidence of cavernous nerve preservation

AJ Bella$^{1,2}$, JF Iaci$^3$, E Coderre$^2$, LP Renaud$^{1,2}$, and AO Caggiano$^3$

University of Ottawa, Canada$^1$, Ottawa Hospital Research Institute, Ottawa, Canada$^2$, ACORDA Therapeutics, NY, USA$^3$

A post-radical prostatectomy neuroprotective and nerve regenerative candidate?

- GGF2 targets nerve injury, therefore allows for potential of remote-from-site delivery

- Schwann cell presence is fundamental to successful axon regeneration
  - Myelination of fibers
  - Trophic support

- GGF2, a human recombinant protein (splice variant of the neuregulin-1 gene) has mitogenic effects on Schwann cells in addition to possible neuroprotective effects

- Receptor binding portion is EGF-like domain, therefore candidate for cavernous nerve injury modulation
Cavernous Nerve Crush Rat Model-Functional Improvements

- Model of erectile dysfunction following prostatectomy
- Intercavernosal pressure at 5 weeks post crush measured after electrostimulation of the crushed nerves
- GGF2 enhanced function in a dose dependent manner, drug delivered subcutaneously before and following crush injury

Flourogold Retrograde Labeling of the MPG

(A) Normal  (B) Crush  (C) Crush + GGF2
Flourogold Retrograde Labeling of the MPG

![Bar graph showing Major Pelvic Ganglia Cell Counts after Retrograde Flourogold Labeling.]

Neuronal nitric oxide synthase (nNos) nerve fiber staining of cavernous nerves in the corpora

(A) Normal  (B) Crush  (C) Crush + GGF2
Vesicular acetylcholine transporter (VaChT) staining of the cavernous nerves in the corpora

(A) Normal  (B) Crush  (C) Crush + GGF2

GGF2 - A post-radical prostatectomy neuroprotective and nerve regenerative candidate

- Remote to site-of-injury delivery of GGF2 – attractive translational candidate in context of prostate cancer treatment
- Functional data supports neuroregenerative and neuroprotective effect
- GGF2 confers recovery advantage to peripheral nerves ** (intact parasympathetic neurons) as neural tissues proximal (MPG) and distal (end organ – penile corpora) to crush injury are preserved

**Ref: ACOR.P0207US - U.S. Patent Appl. No. 12/904,891 Caggiano, Bella, Iaci “Use of a neuregulin to treat a peripheral nerve injury”**
OPTIMIZING ENDOGENOUS RESPONSE

THE NEUROBIOLOGICAL RESPONSE TO BILATERAL Cavernous NERVE INJURY IS MODIFIED BY INTERMITTENT CALORIC RESTRICTION AND FACILITATES RECOVERY OF ERECTILE FUNCTION IN THE AGED RAT

AJ Bella\textsuperscript{1,2}, R Payne\textsuperscript{1}, W Plunet\textsuperscript{4}, JJL Carson\textsuperscript{3}, R Shamloul\textsuperscript{1}, I Cagiannos\textsuperscript{1}, W Tetzlaff\textsuperscript{4} and LP Renaud\textsuperscript{2}

\textsuperscript{1}Division of Urology, Department of Surgery University of Ottawa, Canada
\textsuperscript{2}Department of Neuroscience, University of Ottawa, Canada
\textsuperscript{3}University of Western Ontario, Canada
\textsuperscript{4}and ICORD University of British Columbia, Canada
PURPOSE

• To determine whether every-other-day-fasting (EODF), a form of intermittent caloric restriction, conferred an erectile recovery advantage in the aged rat

• Focus specifically on CN biology

EVERY-OTHER-DAY FASTING (EODF)

• Recent studies suggest that EODF enhances neuroprotection and plasticity of intact fibers in central nervous system injury models
  – modulating inhibitory proteins
  – limiting secondary damage
  – optimizing the intrinsic axonal growth response
CALORIC RESTRICTIONS IMPROVE NEURONAL RECOVERY?

• Dietary restriction has been repeatedly shown to decrease excessive inflammatory changes responsible for progressive nerve damage
  – downregulation of nuclear factor kappa B (NFkB)
  – subsequent decreases of pro-inflammatory molecules TNF-alpha, IL-6, and COX-2

STUDY DESIGN

- 45 18-month old rats
- bilateral nerve crush injury
- Normal Diet
- EODF 2-weeks pre-op ($t_{-2}$)
- EODF immediately post-op ($t_{0}$)
- EODF 1-week post-op ($t_{+1}$)
**STUDY DESIGN**

- 5 weeks post-op all rats underwent intracavernosal pressure (ICP) measurements

- Tissues for immunohistochemical and quantitative studies (cavernous nNOS WB)

- Nangle and Keast – vesicular acetylcholine transporter (VaChT), nNOS, and tyrosine hydroxylase

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**MEASUREMENT OF INTRACAVERNOUS PRESSURE FOLLOWING ELECTROSTIMULATION OF CAVERNOUS NERVES AT 5 MONTHS**

**ICP RESPONSE**

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<th>Group</th>
<th>ICP/MAP (X±SD)</th>
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<td>2 weeks prior</td>
<td>.26 (0.06)</td>
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### MEASUREMENT OF INTRACAVERNOUS PRESSURE FOLLOWING ELECTROSTIMULATION OF CAVERNOUS NERVES AT 5 MONTHS

#### ICP RESPONSE

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![Graph of ICP response](image)
NEURONAL NITRIC OXIDE (nNOS)

- nNOS is primary neurotransmitter of erectile function
- Left images – no injury
- Right image – crush and 2 weeks prior treatment
- Molecular confirmation in progress to confirm EODF treatment advantage
NEURONAL NITRIC OXIDE (nNOS)

- nNOS is primary neurotransmitter of erectile function
- Left images – no injury
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- Molecular confirmation in progress to confirm EODF treatment advantage

COMPARISON BETWEEN STUDIES

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**Note:** ICP/MAP standardized to mean arterial pressure following electrical stimulation.
CONCLUSIONS

- Caloric restriction (EODF) confers functional preservation advantage – neuromodulation – decreased effect in aged rats

- End-organ innervation preserved if EODF initiated 2 weeks prior to injury > at injury

CONCLUSIONS

- Advantages
  - ease of translation to human model
  - no undesired oncogenic side effects
  - cost and complimentary to other strategies
CHARACTERISTICS OF CBT APPROACH

- Pluripotent - self renewal and wide differentiation into multiple lineages
- Can be incorporated directly into tissues
- Readily *ex vivo* expanded, efficiently gene engineered *ex vivo*
- Do not elicit immune rejection (transplanted allogenic MSC are not rejected)

FUTURE OF ED THERAPIES: CBT

- Cell based: MSC (ex. ADSCs) differentiate in penis
  - Cavernosal smooth muscle cells
  - Endothelial cells
- Growth factor based: neurotrophins, cytokines or growth factors secreted from ADSCs
  - Recruit stem cells and activate differentiation
  - Neurotrophic factors promote nerve regeneration
  - iNOS, neurturin, IGF increase
  - BDNF end organ decrease
TARGETS FOR CBT THERAPY?

THE INITIAL CONCEPT: BUILDING BLOCK THEORY (MSC STAY AND DIFFERENTIATE)

• When implanted to damaged tissue, MSCs differentiate to become the new building blocks of the host tissue
ADSCs CAN SURVIVE IN THE PENIS AFTER INJECTION – BUT MOST DO NOT

IHC for BrdU -
Arrows indicate the BrdU (+) cells in brown

CONTEMPORARY CONCEPT:
PROMOTE AND REPAIR THEORY
(PARACRINE FUNCTION)
• When introduced to a ‘damaged tissue’, MSCs and neighboring cells secrete cytokines and chemokines to build new blood vessels, nerves and repair damage
CONTEMPORARY CONCEPT: PROMOTE AND REPAIR THEORY (PARACRINE FUNCTION)

- When introduced to a ‘damaged tissue’, MSCs and neighboring cells secrete cytokines and chemokines to build new blood vessels, nerves and repair damage.

- When work is completed, MSCs move to bone marrow or perivascular area to become reserve cells.

(Dr Lue – cells go home)
Injections of Adipose Tissue-Derived Stem Cells and Stem Cell Lysate Improve Recovery of Erectile Function in a Rat Model of Cavernous Nerve Injury

Maarten Albersen, MD,* Thomas M. Fandel, MD,* Gutting Lin, MD, PhD,* Guifang Wang, MD,* Lia Banie, BS,* Ching-Shwun Lin, PhD,* and Tom F. Luo, MD* J Sex Med 2010;7:3331–3340

Aim. To determine the effects and to identify the mechanism of action of ADSC and ADSC-derived lysate in a rat model of cavernous nerve (CN) crush injury.

Methods. Thirty-two male Sprague-Dawley rats were randomly divided into four equal groups: one group underwent sham operation, while three groups underwent bilateral CN crush. Crush-injury groups were treated at the time of injury with intracavernous injection of ADSC, lysate, or vehicle only (injured controls). Erectile function was assessed by CN electrostimulation at 4 weeks. Penile tissue was collected for histology.

Main Outcome Measures. Intracavernous pressure increase upon CN stimulation; neuronal nitric oxide synthase (nNOS) content in the dorsal penile nerve; smooth muscle content, collagen content, and number of apoptotic cells in the corpus cavernosum.

Results. Both ADSC and lysate treatments resulted in significant recovery of erectile function, as compared with vehicle treatments. nNOS content was preserved in both the ADSC and lysate groups, with significantly higher expression, compared with vehicle-treated animals. There was significantly less fibrosis and a significant preservation of smooth muscle content in the ADSC and lysate groups compared with injured controls. The observed functional improvement after lysate injection supports the hypothesis that ADSCs act through release of intracellular pro-nitric substances or by active secretion of certain biomolecules. The underlying mechanism of recovery appears to involve neuroprotection and apoptosis by inhibition of apoptosis.

Cavernous Nerve Injury (CNI) - Model A

Major Pelvic Ganglion (MPG)  Left CN

Major Pelvic Ganglion (MPG)  Right CN

Cavernous Nerve Injury with forceps 15 sec x 3

Courtesy of T Bivalacqua
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

Recruitment of Intracavernously Injected Adipose-Derived Stem Cells to the Major Pelvic Ganglion Improves Erectile Function in a Rat Model of Cavernous Nerve Injury

Thomas M. Fandell¹,², Maarten Albersen³,⁴, Guiting Lin⁵, Xuefeng Qiu⁵,⁶, Hongxia Ning⁶, Lia Banie⁶, Tom F. Lue⁷, Ching-Shawn Liu⁸,⁹

European Urology 61 (2012) 201–210

Background: Intracavernous (IC) injection of stem cells has been shown to ameliorate cavernous-nerve (CN) injury-induced erectile dysfunction (ED). However, the mechanisms of action of adipose-derived stem cells (ADSC) remain unclear.

Objectives: To investigate the mechanism of action and fate of IC injected ADSC in a rat model of CN crush injury.

Design, setting, and participants: Sprague-Dawley rats (n = 110) were randomly divided into five groups. Thirty-five rats underwent sham surgery and IC injection of ADSC (n = 25) or vehicle (n = 10). Another 75 rats underwent bilateral CN crush injury and were treated with vehicle or ADSC injected either IC or in the dorsal penile perineural space: A, 1, 3, 7 (n = 5), and 28 d (n = 10) postinjury, penile tissues and major pelvic ganglia (MPG) were harvested for histology. ADSC were labeled with 5-ethyl-2-deoxyuridine (EdU) before treatment. Rats in the 28-d groups were examined for erectile function prior to tissue harvest.

Measurements: IC, penile recording: CN; Electrostimulation, immunohistochemistry of the penile and the MPG, and number of EdU-positive (EdU+) cells in the injection site and the MPG.

Results and limitations: IC, but not perineural, injection of ADSC resulted in significantly improved erectile function. Significantly more EdU+ ADSC appeared in the MPG of animals with CN injury and IC injection of ADSC compared with those injected perineurally and those in the sham group. One day after crush injury, strong cell-derived factor-1 (SDF-1) was upregulated in the MPG, providing an incentive for ADSC recruitment toward the MPG. Neovascularization was observed in the group that underwent IC injection of ADSC, and IC, ADSC treatment had beneficial effects on the smooth muscle cell proliferation in the corpus cavernosum.

Conclusions: CN injury upregulates SDF-1 expression in the MPG and thereby attracts intracavernously injected ADSC. At the MPG, ADSC exert neuroregenerative effects on the cell bodies of injured nerves, resulting in enhanced erectile response.
FUTURE OF ED THERAPIES: CBT

• www.clinicaltrials.gov

• ‘Evaluate the Use of Liposuction and Cell Separation Devices for Autologous Fat (Adipose) Derived Cells to Treat the Symptoms of Erectile Dysfunction’ - American Medical Systems

• In other words…ADSCs for the treatment of erectile dysfunction

• First Received on May 9, 2012. Last Updated on May 16, 2012.
• ClinicalTrials.gov processed this record on May 17, 2012

• ClinicalTrials.gov Identifier: NCT01601353

Primary Outcome Measures:
Adverse Events that occur during or after the procedure to measure safety and tolerability
Erectile function

Secondary Outcome Measures [3 years]:
Continence
Treatment assessment
Erection hardness

Estimated Enrollment: 30
Study Start Date: May 2012
Estimated Study Completion Date: May 2016
Estimated Primary Completion Date: May 2016 (Final data collection date for primary outcome measure)
OUTLINE

- Identify the translational need for optimizing recovery of cavernous nerve injury
- Review nerve cell and neurovascular bundle function in this clinical context
- Outline select research programs with translational potential for this patient group

The Molecular Mechanisms of Cavernous Nerve Response to Injury at Prostate Cancer Surgery: Identifying Translational Targets to Impact Patient Care

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