Cell versus Chemokine Therapy in a Nonhuman Primate Model of Chronic Intrinsic Urinary Sphincter Deficiency

J. Koudy Williams,* Ashley Dean, Sherif Badra, Shannon Lankford, Kimberly Poppante, Gopal Badlani and Karl-Erik Andersson

From the Wake Forest Institute for Regenerative Medicine (JKW, AD, SL, KP, KEA) and Department of Urology (GB), Wake Forest Baptist Medical Center, Winston-Salem, North Carolina, Urology Department, Ain-Shams University Hospitals (SB), Cairo, Egypt, and Institute for Clinical Sciences, Department of Obstetrics and Gynecology, Aarhus University (KEA), Aarhus Denmark

Purpose: Mixed efficacy results of autologous skeletal muscle precursor cell therapy in women with chronic intrinsic urinary sphincter deficiency have increased interest in the therapeutic value of alternative regenerative medicine approaches. The goal of this study was to compare the effects of the cell homing chemokine CXCL12 (C-X-C motif chemokine 12) and skeletal muscle precursor cells on chronic urinary sphincter regeneration in chronic intrinsic urinary sphincter deficiency.

Materials and Methods: Five million autologous skeletal muscle precursor cells or 100 ng CXCL12 were injected in the urinary sphincter complex of adult female cynomolgus monkeys with chronic (6-month history) intrinsic urinary sphincter deficiency. These treatment groups of 3 monkeys per group were compared to a group of 3 with no intrinsic urinary sphincter deficiency and no injection, and a group of 3 with intrinsic urinary sphincter deficiency plus vehicle injection. Maximal urethral pressure was measured at rest, during stimulation of the urinary sphincter pudendal nerves at baseline and again 6 months after treatment. The monkeys were then necropsied. The urinary sphincters were collected for tissue analysis of muscle and collagen content, vascularization and motor endplates.

Results: CXCL12 but not skeletal muscle precursor cells increased resting maximal urethral pressure in nonhuman primates with chronic intrinsic urinary sphincter deficiency compared to that in monkeys with intrinsic urinary sphincter plus vehicle injection ($p > 0.05$). Skeletal muscle precursor cells and CXCL12 only partially restored pudendal nerve stimulated increases in maximal urethral pressure ($p > 0.05$), sphincter vascularization and motor endplate expression in monkeys with chronic intrinsic urinary sphincter deficiency. Additionally, CXCL12 but not skeletal muscle precursor cell injections decreased collagen and increased the muscle content of urinary sphincter complex in monkeys with chronic intrinsic urinary sphincter deficiency compared to those with intrinsic urinary sphincter plus vehicle injection and no intrinsic urinary sphincter plus no injection ($p < 0.05$ and > 0.05, respectively).

Conclusions: These results raise questions about cell therapy for chronic intrinsic urinary sphincter deficiency and identify a chemokine treatment (CXCL12) as a potential alternative treatment of chronic intrinsic urinary sphincter deficiency.

Key Words: urinary bladder; urinary incontinence, stress; myoblasts; chemokine CXCL12; cell- and tissue-based therapy

Abbreviations and Acronyms

CXCR4 = chemokine receptor type 4

DMEM = Dulbecco's modified Eagle's medium

ISD = intrinsic urinary sphincter deficiency

MSC = mesenchymal stem cell

MUP = maximal urethral pressure

NHP = nonhuman primate

skMPC = skeletal muscle precursor cell

SUI = stress urinary incontinence

Accepted for publication May 25, 2016.

No direct or indirect commercial incentive associated with publishing this article.

The corresponding author certifies that, when applicable, a statement(s) has been included in the manuscript documenting institutional review board, ethics committee or ethical review board study approval; principles of Helsinki Declaration were followed in lieu of formal ethics committee approval; institutional animal care and use committee approval; all human subjects provided written informed consent with guarantees of confidentiality; IRB approved protocol number; animal approved project number.

Supported by R01 DK 083688 (Regeneration of the Lower Urinary Tract in Nonhuman Primates).

* Correspondence: Wake Forest Institute for Regenerative Medicine, Wake Forest University. 391 Technology Way, Winston-Salem, North Carolina 27101 (telephone: 336-713-1323; FAX: 336-713-7290; e-mail: kwilliam@wakehealth.edu).

0022-5347/16/1966-0001/0
THE JOURNAL OF UROLOGY®
© 2016 by American Urological Association Education and Research, Inc.
http://dx.doi.org/10.1016/j.juro.2016.05.106
Vol. 196, 1-7, December 2016
Printed in U.S.A.

UNCORRECTED PROOF

GOOD results of surgical therapy of SUI have been reported.[1] However, complications are not infrequent[2] and alternative treatments may be desirable, particularly when surgical treatment has failed or surgery poses too great a risk. Studies using adult MSCs to induce tissue regeneration and repair of the damaged urethral sphincter have shown promising results in animals[3] and humans.[4] However, the efficacy of cell therapies in clinical studies appears to be modest (around 50% improvement in 50% of patients) and it has increased interest in alternative or adjunct regenerative medicine therapies for ISD.

Progenitor cells produce and release an array of bioactive molecules, the secretome, which comprises a host of diverse cytokines, chemokines, angiogenic factors and growth factors.[5] These secretomes have been tested as treatment options for lower urinary tract dysfunctions in animals, including for ISD.[6,7] One of the factors of interest is CXCL12, also called SDF-1 (stromal derived factor-1), which has a major role in cell trafficking and homing of progenitor cells to sites of injury through a receptor (CXCR4) mechanism and enhancing cell survival once at the injury site.[8] In a NPH model of urethral sphincter dysfunction we previously found that skeletal muscle MSCs could prevent functional and structural changes when injected within 6 weeks after inflicted sphincter injury.[9] We also observed that the sphincter injury without treatment was irreversible for up to 12 months. Clinically, preventing SUI development with cell therapy is currently not feasible and most women with ISD have a chronic condition.

To mimic the clinical situation in the current study we compared the ability of skeletal muscle MSCs and CXCL12 to restore sphincter function and structure in NHPs with chronic ISD.

METHODS

Animal Model

Studies in these nonhuman primates were approved by the Wake Forest University institutional animal care and use committee and performed in compliance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals. Sacrifice was performed according to AVMA (American Veterinary Medical Association) standards.

Design

In this study 12 adult female cynomolgus monkeys 5 to 10 years old were used. All monkeys had experimentally induced ISD as described by Badra et al.[9,10] Three monkeys served as no ISD-no treatment controls. In 9 monkeys ISD was surgically created. Six months following the ISD procedure 3 monkeys received 100 ng CXCL12 local injections, 3 received local injections of 5 million autologous skMPCs and 3 received only vehicle injections. The vehicle solution was DMEM without serum. MUP was measured before the ISD procedure, before injections and at 3-month intervals after injections. Six months after injection (1 year after the ISD procedure) the NHPs were sacrificed and tissues were collected.

Nonhuman Primate Instrinsic Urinary Sphincter Deficiency Model

The ISD procedure was done after baseline urodynamic measures were obtained. The monkeys were sedated with 10 to 15 mg/kg ketamine intramuscularly and with 1% to 5% isoflurane used for anesthesia induction and maintenance. The monkeys were prepared for aseptic surgery and anesthetized. A lower midline abdominal incision 4 cm long was made to expose the pelvic area of the abdomen. The distal urinary tract was approached using gentle dissection of connective tissue just ventral to the bladder, extending dorsal to the bladder neck and caudal 2 cm to each side of the rhabdosphincter. The pudendal nerve branches supplying the sphincter (usually 3) were identified, selectively electrocauterized while not damaging the sphincter directly, and then transected.[9,10] Special care was taken not to damage surrounding structures. The abdomen was closed in 2 layers and postoperative support was given.

skMPC and CXCL12 Injections

A 1 cm^3 sample of quadriceps muscle was aseptically removed from an anesthetized NHP and transported in a wash solution of Dulbecco's phosphate buffered saline (HyClone™) with 1% antibiotic/antimitotic (HyClone). The tissue was washed for 10 minutes × 3 in fresh wash solution with periodic gentle agitation with a final rinse in Dulbecco's phosphate buffered saline. The sample was trimmed of unwanted tissue, weighed and minced into fragments approximately 0.5 mm^2 or less. Digestion medium consisted of 2:1 dispase II (Sigma Aldrich®) to collagenase type I (Worthington, Lakewood, New Jersey) per ml basal medium, which was custom designed muscle progenitor cell medium (PeproTech, Rocky Hill, New Jersey). The digestion medium was added to the minced tissue at 1 ml/100 mg tissue. The sample was incubated at 37C in 5% CO_2 for 45 minutes. Upon completion digestion was terminated using 2 × volume of growth medium (PeproTech basal medium plus fetal bovine serum and custom growth supplements) to digestion medium and rigorous pipetting was applied.

The suspension was filtered through a 100 μ filter and centrifuged for 5 minutes at 1,500 rpm. The supernatant was aspirated and fresh growth medium was added and spun for a second time. The sample was then plated on a pretreated collagen-I 100 mm BD Biocoat™ culture plate and incubated for 24 hours at 37C in 5% CO_2. The following day the aspirate was collected and replated on a new pretreated collagen coated plate to reduce fibroblasts in the cell culture.

The skMPCs were isolated and characterized as described previously.[9] Eight weeks after sample collection 5 million skMPCs were suspended in 2 ml DMEM without serum plus 1.067 mg/ml rattail collagen (type 1 collagen, GIBCO™) to assist with tissue retention of the injected

UNCORRECTED PROOF

Download English Version:

https://daneshyari.com/en/article/5687300

Download Persian Version:

https://daneshyari.com/article/5687300

Daneshyari.com