



Efficacy and Initial Safety Profile of CXCL12 Treatment in a Rodent Model of Urinary Sphincter Deficiency

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Key Words. Intrinsic urinary sphincter deficiency • Skeletal muscle precursor cells • Stress urinary incontinence • Stromal derived factor-1 α • CXCL12

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ABSTRACT

Disappointing results of skeletal muscle precursor cell (skMPC) therapy for women with intrinsic urinary sphincter deficiency (ISD) associated urinary incontinence has increased interest in alternative sphincter regenerative approaches. This study was to measure the safety and efficacy of the cell homing chemokine CXCL12 versus skMPCs in a rat model of ISD. Thirty-six adult female Sprague Dawley rats were divided into 6 treatment (Tx) conditions: (a) no ISD/noTx [Control]; (b) ISD/noTx; (c) ISD + skMPCs; (d) ISD + 3.5 mg CXCL12; (e) ISD + 7mg CXCL12; and (f) ISD + 14 mg CXCL12. Tx's were injected directly into the sphincter complex 30 days post ISD and rats euthanized 30 days post Tx. Blood samples for measurements of kidney and liver function, white and red blood cell counts, were taken at baseline and at euthanasia. Leak point pressures (LPP) were measured prior to, and sphincter collagen/muscle content measured after, euthanasia. There were no effects of treatments on white or red/white blood cell counts, kidney/liver function tests or histopathology of the urinary sphincter complex or surrounding tissues. ISD lowered LPP 35% and sphincter muscle content by 17% versus control rats. CXCL12, but not skMPC injections, restored both LPP to control values in a dose-dependent fashion. Both skMPCs and CXCL12 restored sphincter muscle content to control values. This chemokine approach may represent a novel therapeutic option for ISD and appears, at least short-term, to produce little clinical or tissue pathology. *STEM CELLS TRANSLATIONAL MEDICINE* 2017;6:1740–1746

SIGNIFICANCE STATEMENT

Cell therapy for urinary incontinence has been only moderately effective. This study reports the effectiveness and safety of a new method of treating urinary incontinence by injecting a chemical that stimulates the body to heal itself.

INTRODUCTION

Intrinsic urinary sphincter deficiency (ISD) is a common cause of stress urinary incontinence (SUI) and remains a significant quality of life issue. It is a chronic condition resulting from aging and childbirth injury to the urinary sphincter musculature and innervation becoming clinically evident in the peri/postmenopausal years [1, 2]. Although good results of surgical therapy of SUI have been reported [3], complications are not infrequent [4] and alternative treatments may be desirable, particularly when surgical treatment has failed or if surgery poses too great a risk. Studies using adult stem cells to induce tissue regeneration and repair of the damaged urethral sphincter have shown promising results both in animals [5] and humans [6, 7]. However, the efficacy of cell therapies in clinical studies appear to be modest (around 50% improvement in 50% of patients) and has increased interest in alternative or adjunct regenerative medicine therapies for ISD.

Progenitor cells produce and release an array of bioactive molecules, which include a host of diverse cytokines, chemokines, angiogenic factors, and growth factors [8, 9]. One of the factors of interest is CXCL12 (also called stromal derived factor-1 = SDF-1) which plays 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 [10]. We have reported that local injection of CXCL12 promotes regeneration of sphincter structure and function in nonhuman primates both when given at the creation of ISD and administered to animals with established chronic ISD [11]. However, there is some concern that administration of CXCL12 may increase the risk of fibrotic diseases in the lung [12], inflammation [13] and possibly increase the risk of cancer [14]. In fact, there are current anti-cancer therapies designed to inhibit the CXCL12/CXCR4 axis [14]. This study provides information about the dose-effects of CXCL12 on efficacy and safety in a rodent model of ISD.

MATERIALS AND METHODS

Animal Model

This study utilized 36 adult female Sprague Dawley rats. All procedures done on these rats were approved by the Wake Forest Institutional Animal Care and Use Committee (IACUC) (protocol # A13-130) and done in compliance with the Animal Welfare Act, and the Guide for Care and Use of Laboratory Animals. All animals were euthanized according to American Veterinary Medical Association guidelines.

Study Design

The adult female rats were divided into six experimental conditions and received: (a) Control—no ISD procedure and no treatment; (b) the ISD procedure, but no treatment; (c) the ISD procedure + sphincter injection of 5×10^6 autologous skeletal muscle precursor cells (skMPCs) collected from a biopsy of the quadriceps muscle; (d) the ISD procedure + sphincter injection of 3.5 ng CXCL12; (e) the ISD procedure + sphincter injection of 7.0 ng CXCL12; or (f) the ISD procedure + sphincter injection of 14 ng CXCL12. All local injections were 0.5 ml in volume and consisted of the drug/cells suspended in basal DMEM without serum. All rats received a muscle biopsy to equalize groups for any regenerative response initiated by the biopsy procedure. The biopsies were done at the same time as the ISD procedure. Injections were done 30 days post ISD and the rats euthanized 30 days post treatment. The dose of the skMPCs was based on the results of study done by You et al. [15] who achieved sphincter regeneration in rats receiving the amount of skMPCs. The dose of CXCL12 was based on the results of our nonhuman primate model where 100 ng restored sphincter structure and function after ISD [11]. The weight of the urinary sphincter of the rats in this study averages 100 mg. The average weight of a monkey urinary sphincter is around 1.5 gm. Therefore, 7 ng for a rat is equivalent to the 100 ng dose given to the monkeys. We chose this dose, half the dose and twice the dose for our study.

ISD Procedure

Rats were anesthetized with isoflurane and prepared for aseptic surgery of their abdomen as described in our IACUC protocol. A midline abdominal incision (approximately 1 cm in length) was made from a point distal to the umbilicus to the pubis. The urinary bladder was exteriorized and the vasculature and innervation dissected away from the bladder, extending to the pelvic outlet. This tissue was cauterized to prevent re-growth. Additionally, the urethra from the bladder neck to the pelvic outlet, was hydro-dissected to remove the outer layer of the urethral skeletal muscle. This was done by injecting 1.0 ml into the muscular layer to separate it from the remaining urethral wall and the outer muscle layer carefully dissected away. The abdominal wall and the skin were closed and the rats returned to their cage.

skMPC and CXCL12 Injections

A 2 mm² sample of quadriceps muscle was aseptically removed from anesthetized rats and transported in a wash solution of Dulbecco's Phosphate Buffered Saline (DPBS, HyClone, South Logan UT) with 1% antibiotic/antimitotic (HyClone). The tissue was washed 10 min \times 3 in fresh wash solution with periodic gentle agitation with a final rinse in DPBS. The sample was trimmed of unwanted tissue, weighed, and minced into fragments approximately 0.5 mm² or less. Digestion media consisting of 2:1 dispase

II (Sigma Aldrich, St. Louis MO): collagenase type I (Worthington, Lakewood NJ) per ml of basal media (custom designed muscle progenitor cell media, PeproTech, Rocky Hill NJ) was added to the minced tissue at 1 ml per 100 mg of tissue. The sample was incubated at 37°C, 5% CO₂ for 45 minutes. Upon completion, the digestion was terminated using 2 \times volume of growth media (PeproTech basal media plus FBS and custom growth supplements) to digestion media and rigorous pipetting was applied. The suspension was filtered through a 100 micron filter and centrifuged for 5 minutes at 1,500 rpm. The supernatant was aspirated, fresh growth media was added, and spun for a second time. Then the sample was plated on a pretreated collagen-I 100 mm culture plate (BD Biocoat, Becton, Dickinson, Franklin Lakes NJ) and incubated for 24 hours at 37°C, 5% CO₂. The following day, the aspirate was collected and re-plated on a new pretreated collagen coated plate to reduce fibroblast in the cell culture. The skMPCs were isolated and characterized as described previously [9]. Four weeks following collection of the sample, one million passage 3 skMPCs were suspended in 0.5 ml of DMEM without serum and injected directly into the urinary sphincter complex (at the level of the sphincter skeletal muscle layer and at 4 locations (12, 3, 6, and 9 o'clock positions). For those rats receiving the chemokine injections, 3.5, 7.0, or 14 ng of human recombinant CXCL12 was suspended in 0.5 ml of DMEM and injected in an identical fashion to the skMPCs. ISD rats not receiving treatment had 0.5 ml DMEM only injected into their urinary sphincters. The treatments were directed to the proximal 1/2 of the sphincter length.

Body Weights/Collection of Blood for Cell Counts and Kidney/Liver Enzymes

Body weights and blood samples were taken on all rats prior to any procedures and again at necropsy. Similarly, 1 ml of blood was collected from the saphenous vein into (EDTA tubes and serum separators) heparinized tubes and plasma used for analysis of red and white blood cell counts using ADVIA 120 Hematology System (Siemens, Terrytown, NY), hemoglobin, blood urea nitrogen (BUN), creatinine, alanine transaminase (ALT), and aspartate aminotransferase (AST) using (SYNCHRON Clinical Systems CX5CE (Fullerton, CA).

Leak-Point Pressure Measurements

At the end of their treatment period, rats were anesthetized with isoflurane, placed on an incline table and a midline abdominal incision made to expose the urinary bladder. Leak point pressure (LPP) and closing pressure (CP) were measured using the vertical tilt/intravesical pressure clamp model. Before measuring, the spinal cord was transected at the T9 to T10 or T10 to T11 level to eliminate reflex bladder activity in response to increasing intravesical pressure. This supra-sacral spinal cord transection does not interfere with the spinal continence reflexes of the bladder neck and urethra. The skin was closed with suture or staples. Under general anesthesia, the bladder was exposed by a midline incision. One transvesical catheters with a fire-flared tip was inserted into the dome of the bladder and the abdominal wall and a suture or glue was used to keep the tubing in place. The overlying skin was closed with sutures or staples. The rats were then mounted on a tilt table and placed in the vertical position. The intravesical pressure was measured by connecting a syringe reservoir to one bladder catheter. The intravesical pressure was increased in 1- to 3-cm H₂O steps from 0 cm H₂O upward until visual identification of the leak point height. The pressure at this leak point was referred to as the LPP. The intravesical pressure was decreased in 1- to 3-cm

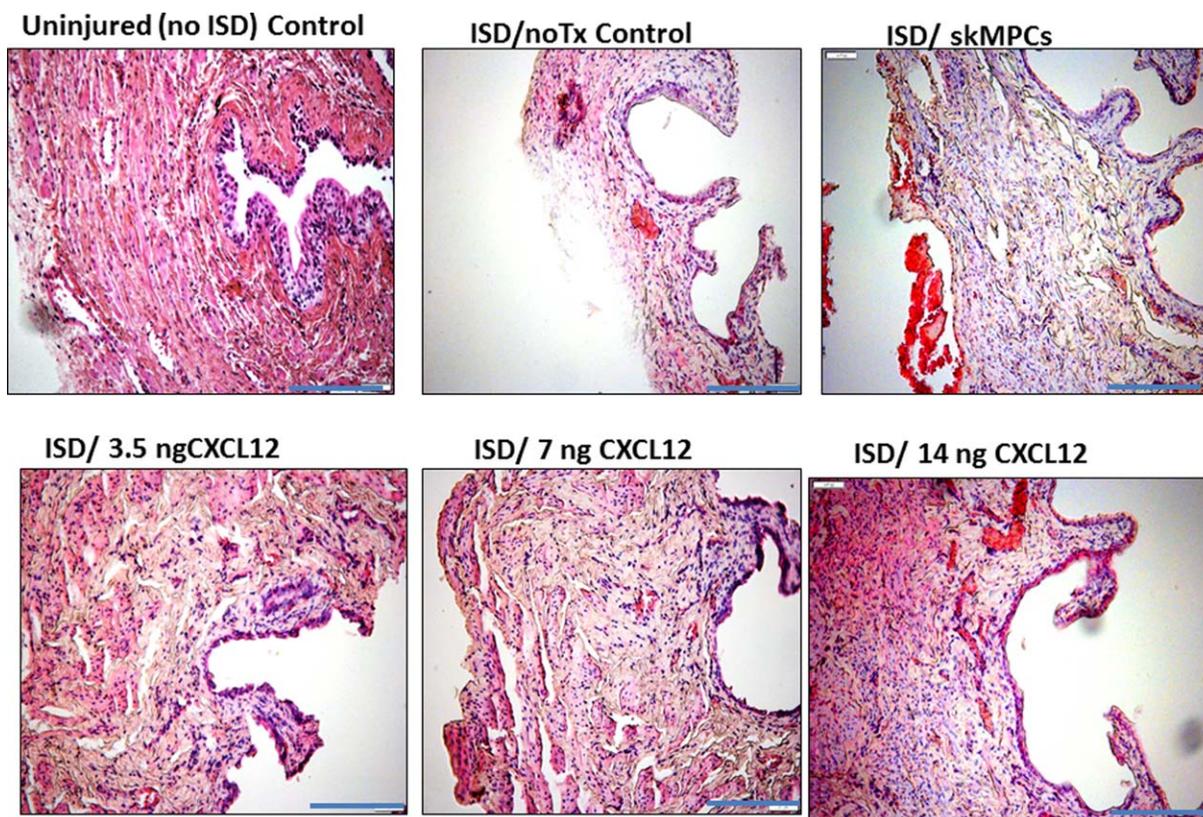


Figure 1. Hematoxylin and Eosin (H&E) staining of the proximal urinary sphincter complex of the treatment groups. The groups are: (1) No ISD procedure and no Tx; (2) rats receiving the ISD procedure, but noTx; (3) Rats receiving the ISD procedure and an intra-sphincter injection of skMPCs (5 million) directly into their urinary sphincter complex 30 days post ISD procedure; (4) rats with the ISD procedure and receiving 3.5 ng CXCL12 direct injection into the urinary sphincter; (5) rats with ISD and receiving 7.0 ng CXCL12 direct injection; and (6) rats with ISD receiving 14 ng CXCL12 direct injection. The scale bars are 200 μ m. Abbreviations: Tx, treatment; skMPCs: skeletal muscle precursor cells.

H₂O steps downward until the leak ceased. The averages of LPP measurements were taken as data points for each animal. Following the completion of the procedure, the animal was euthanized.

Necropsy and Collection of Tissues

Rats were first heparinized (100 IU/kg IV) and then euthanized using sodium pentobarbital (80–100 mg/kg, IV). Prior to removal of any tissues, a general assessment was made on any visible abnormalities that may be present externally, or internally. The entire urinary sphincter complex was then removed and placed in 4% paraformaldehyde for 48 hours. The sections were then placed in 70% ethanol, and stained with hematoxylin and eosin (H&E). Additionally, sphincter muscle/collagen content was assessed from the proximal $\frac{1}{2}$ of the sphincter complex (the location of the treatment injections) using quantitative histomorphometric analysis of sphincter sections stained with Mason's Trichrome. Immunohistochemistry for Protein Gene Product PGP9.5 was done in triplicate with a serum isotype control. We used Anti-PGP9.5 antibody [13C4/I3C4] from AbCam (ab8189). The antibody was diluted to 1:100. Pictures were taken with Leica DM4000B compound microscope. Sections were blocked using serum free blocking agent (Dako, Carpinteria, CA, USA) to avoid nonspecific binding of primary antibodies. Primary antibody was added on the sections and incubated for 12 hours. Protein expression was identified using Nova Red staining.

The remaining tissue removed at necropsy was placed in 4% neutral buffered formalin, sectioned, and stained with H&E.

Representative sections of urethra, urinary bladder, uterine body, uterine horns, large intestine, regional lymph nodes, liver, spleen, adrenal glands, kidneys, heart, caudal lobe of lungs, and ovaries were collected and assessed in a blinded fashion (slide number only) by Dr. Williams.

Statistical Approach

LPP and sphincter collagen/muscle content data were first analyzed using a one-way ANOVA. Post-hoc analysis was done using unpaired Student's *t* test with Bonferroni correction for multiple group comparisons. Data are presented as mean \pm standard error of the mean. Statistical significance threshold was set at $p < .05$.

RESULTS

Histological Sequelae of the ISD Procedure and Treatments

The images seen in Fig. 1 depict the effects of the ISD procedure and treatment paradigms on general structural sphincter histology as evaluated using Hematoxylin and Eosin (H&E) staining of the proximal sphincter complex (the target area of the ISD procedure). The ISD procedure thinned (dilated) the sphincter and removed the outer muscle layer. Treatment with skMPCs or CXCL12 restored the general architecture, thickness and muscle layers of the sphincter.

LPP

There were significant differences among experimental groups in LPP (ANOVA = 0.03, Table 1). LPP were lower in the ISD/noTx group than the no ISD/noTx (control) group (Table 1, $p < .05$). Injection of skMPCs did not increase LPP back to control values ($p > .05$). However, injections of CXCL12 restored LPP values to control values ($p < .05$ vs. ISD/noTx and $p > .05$ vs. control) with the maximal effect being reached at the mid-CXCL123 dose (7 ng) and no further increase at 14 ng (Table 1).

Sphincter Muscle and Collagen Content

There were significant differences among the experimental groups in sphincter muscle/collagen content (ANOVA = 0.02, Table 2). The collagen content is the reciprocal of the muscle content, so significant effects of muscle are mirrored in the collagen content. Muscle content was lower, and collagen content higher in the

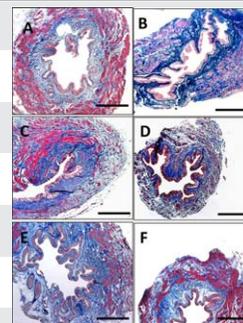
Table 1. Leak point pressures (LPP)

Groups ($n=6$ /group)	Leak point pressure (cmH ₂ O)	SEM
No ISD/No treatment (Tx)	22.8	3.1
ISD/ no Tx	14.8*	1.5
ISD + skMPCs	17.1	2.0
ISD + 3.5 ng CXCL12	22.8#	1.4
ISD + 7.0 ng CXCL12	28.0#	1.7
ISD + 14 ng CXCL12	25.1#	1.0
Statistics	ANOVA $p = .03$ * $p < .5$ vs. Control # $p < .05$ vs. ISD/no Tx	

This table presents the LPP values in the experimental groups of rats. The groups are: (1) No ISD procedure and no treatment (Tx); (2) rats receiving the ISD procedure, but noTx; (3) Rats receiving the ISD procedure and an intra-sphincter injection of skeletal muscle precursor cells (skMPCs) (5 million) directly into their urinary sphincter complex 30 days post ISD procedure; (4) rats with the ISD procedure and receiving 3.5 ng CXCL12 direct injection into the urinary sphincter; (5) rats with ISD and receiving 7.0 ng CXCL12 direct injection; and (6) rats with ISD receiving 14 ng CXCL12 direct injection. There were $n = 6$ rats/experimental group. Values presented are mean \pm SEM. Data first analyzed with ANOVA and then post-hoc with Student's t test. Statistical significance set at $p < .05$.

Table 2. Sphincter muscle/collagen content

Group ($n=6$ /group)	Group average (muscle)	Group average (collagen)
No ISD/NoTx.	62.74 \pm 2.1	37.26 \pm 2.0
ISD/noTx	52.36 \pm 1.9*	48.64 \pm 2.2*
ISD + skMPCs	57.87 \pm 1.7#	42.13 \pm 1.5#
ISD + 3.5 ng CXCL12	49.54 \pm 1.3	50.46 \pm 1.3
ISD + 7.0 ng CXCL12	65.29 \pm 3.7#	44.71 \pm 1.4#
ISDF + 14 ng CXCL12	61.44 \pm 3.4#	38.56 \pm 2.7#
Statistics	ANOVA $p = .02$ * $p < .05$ vs. NoISD/ NoTx # $p < .005$ vs. ISD/noTx	
	ANOVA $p = .02$ * $p < .05$ vs. NoISD/NoTx # $p < .05$ vs. ISD/no Tx	



In these same groups of rats, we measured the sphincter content of muscle and collagen using quantitative histomorphometry of Mason's Trichrome stained slides. The collagen and muscle values are reciprocal of each other. Data were analyzed in the same manner as the LPP values. Values are mean \pm SEM and statistical significance set at $p < .05$. Images A–F are Mason's Trichrome stained sections of the proximal urinary sphincters representing the experimental groups of rats. Red is muscle, Blue is collagen. Scale bars are 200 μ m.

ISD/noTx group than the noISD/noTx (control) group ($p < 0.05$, Table 2). Both skMPC and CXCL12 injections restored sphincter muscle/collagen content to control values ($p > 0.5$ vs. control) and increased sphincter muscle compared to ISD/noTx, ($p < .05$, Table 2). Mirroring the LPP results, the maximum effect of CXCL12 injection was reached at 7 ng, with no further increase at 14 ng (Table 2). Mason's Trichrome images of the sphincter complex representing the different treatment groups are shown in images A–F to the left of the Table. While not dramatic, there is an increase in collagen content (blue) in comparison to muscle (red) in the ISD/noTx group (A vs. B). The different treatments treatment groups, the red (muscle) is restored to near normal content and distribution. This is seen especially in the higher dose of CXCL12 (F).

Innervation

The injury procedure produced a reduction in the amount of PGP9.5 staining seen in the urinary sphincter complex (Fig. 2). The images were not quantified, but there was consistent reduction in the amount of staining throughout the sphincter. The images presented in Fig. 2 are taken just luminal to the skeletal muscle layer. The expression of PGP9.5 (arrows) was somewhat restored in both the skMPCs and the CXCL12-treated groups (Fig. 2). Any potential differences in the expression of PGP9.5 between these two groups were not readily apparent.

Tissue Pathology

The ISD procedure created necrosis and some hemorrhage within the sphincter complex in most all of the rats (Table 3). skMPC injections somewhat reduced the observed necrosis and hemorrhage, but the CXCL12 seemed to do a better job with only a few animals in each of the CXCL12 dose-groups exhibiting sphincter necrosis (Table 3). The numbers of animals in each group were not sufficient to run statistics on these differences. There were adhesions of the bladder to the omentum in many of the animals in each group. There was some miscellaneous pathology (inflammatory cells in the ureters) but no apparent treatment effects on this pathology. There was some uterine inflammation (presence of inflammatory cells) in some of the animals in each group (again, no specific treatment effect), and an enlarged spleen in one of the 35 ng CXCL12 treated rats. There was no histological or gross pathology noted in the kidneys, heart, spleen or liver in any of the other rats in any of the treatment groups.

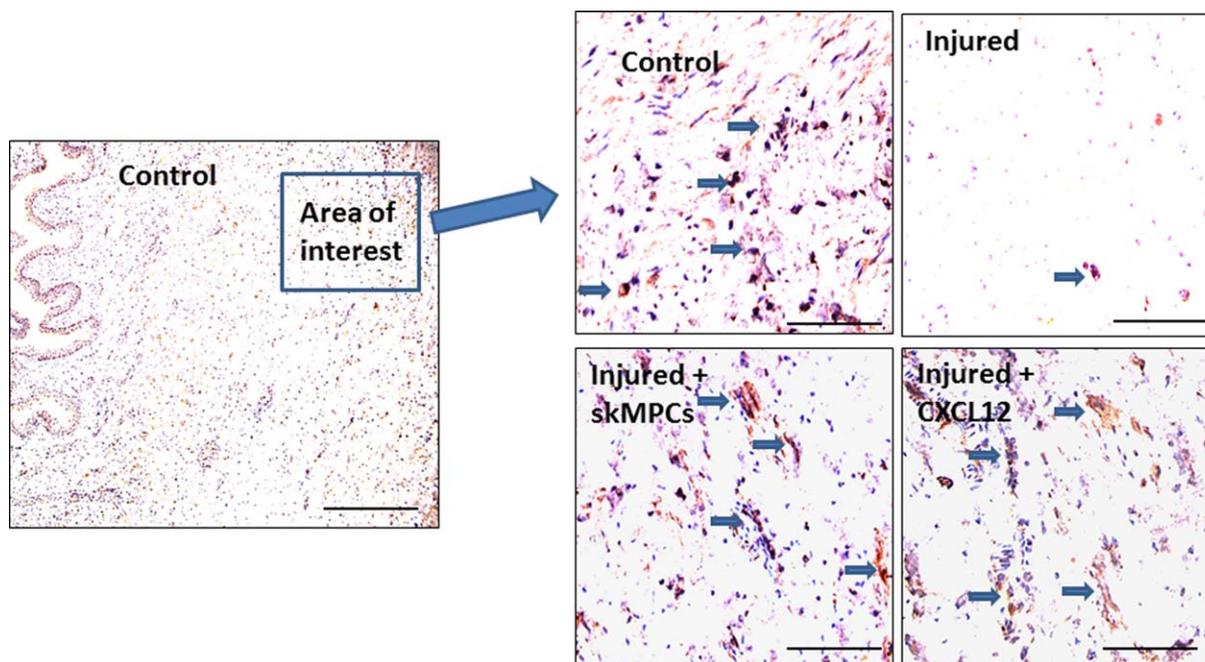


Figure 2. Immunohistochemical sections of rat urethral sphincter stained with the neuronal marker PGP9.5. The figure to the left is a low-power image of where the images on the right were taken within the sphincter. Staining was with Nova red, so positive staining is reddish-brown. PGP9.5 (arrows). Staining is shown for the un-injured control group; the injured group with no treatment; and the injured groups with either skMPCs; or CXCL12 staining. Scale bars are 200 μm for the low power and 100 μm for the higher powered images on the right. Abbreviation: skMPC: skeletal muscle precursor cell.

Table 3. Tissue pathology

Group ($n=6/\text{group}$)	Urethral pathology	Ureter pathology	Bladder pathology	Peripheral pathology
Control	0/6	0/6	0/6	0/6
Injured	5/6 - muscle necrosis, hemorrhage	1/6 - inflammatory cells, enlarged	4/6 - adhesions	1/6 - uterine adhesion
skMPCs	3/6 - muscle necrosis	0/6	4/6 - adhesions	2/6 - uterine inflammation
3.5 ng CXCL12	2/6 - muscle necrosis	0/6	3/6 - adhesions	1/6 - enlarged spleen
7.0 ng CXCL12	1/6 - muscle necrosis	0/6	3/6 - adhesions	1/6 - uterine inflammation

This table depicts the observations made of the sphincter tissue and adjacent tissues and organs both grossly and evaluating hematoxylin and eosin-stained slides in these same groups of rats. Observations were made in a blinded fashion by Dr. Williams.

Blood Results

There were no significant effects of treatment on ALP or AST (ANOVA = 0.42, Fig. 3). Liver function tests (ALP and AST). However, AST trended a little higher at necropsy (Final) ($p = .1$). There was one vehicle-only rat that had very high AST values, which markedly increased the variance. There was no effect of treatment on kidney function tests (BUN and Creatinine (ANOVA = 0.32, Fig. 3). However, both values were higher at necropsy than baseline in all groups ($p < .05$ vs. baseline). It is unclear why this happened in that there was no significant histopathological pathology in the kidneys in any of the groups. There was no effect of treatment on RBC, WBC, HCT, or MCHC values (ANOVA = 0.51, Fig. 4). Furthermore, there was no difference between baseline and necropsy values in any of the groups ($p > .05$, Fig. 4).

DISCUSSION

This study was a direct comparison between a dose of skMPC shown to be effective in restoring urinary sphincter structure and function in animal models with induced intrinsic sphincter

deficiency (ISD) [15], and three increasing doses of CXCL12 that bracket a dose shown to be effective in restoring the urinary sphincter of nonhuman primates with ISD [11]. The goal was to assess any dose effects of the CXCL12 and to determine if CXCL12 has detectable safety issues in this rat model of ISD. The main findings were that: (a) CXCL12 was effective in restoring sphincter structure and function in this model; (b) there was a dose effect of CXCL12 restoration of the urinary sphincter; and (c) there were no effects of treatments (skMPCs or CXCL12) on blood values of liver and kidney function, white blood cell counts, or red blood cell counts.

While CXCL12, but not skMPCs, treatment resulted in higher LPP values, it must be remembered that these measures were done on anesthetized rats. Anesthesia can blunt urinary reflexes, and may have affected the results. However, similar to what we have shown in nonhuman primates [11], the effects of CXCL12 were pronounced and are most likely valid. It is unclear why the dose of skMPCs was not effective on LPPs. The number of cells used has been shown to be effective in previous studies in rats [15]. However, the model of creating ISD was different in the present study and focused on nerve damage, whereas many animal

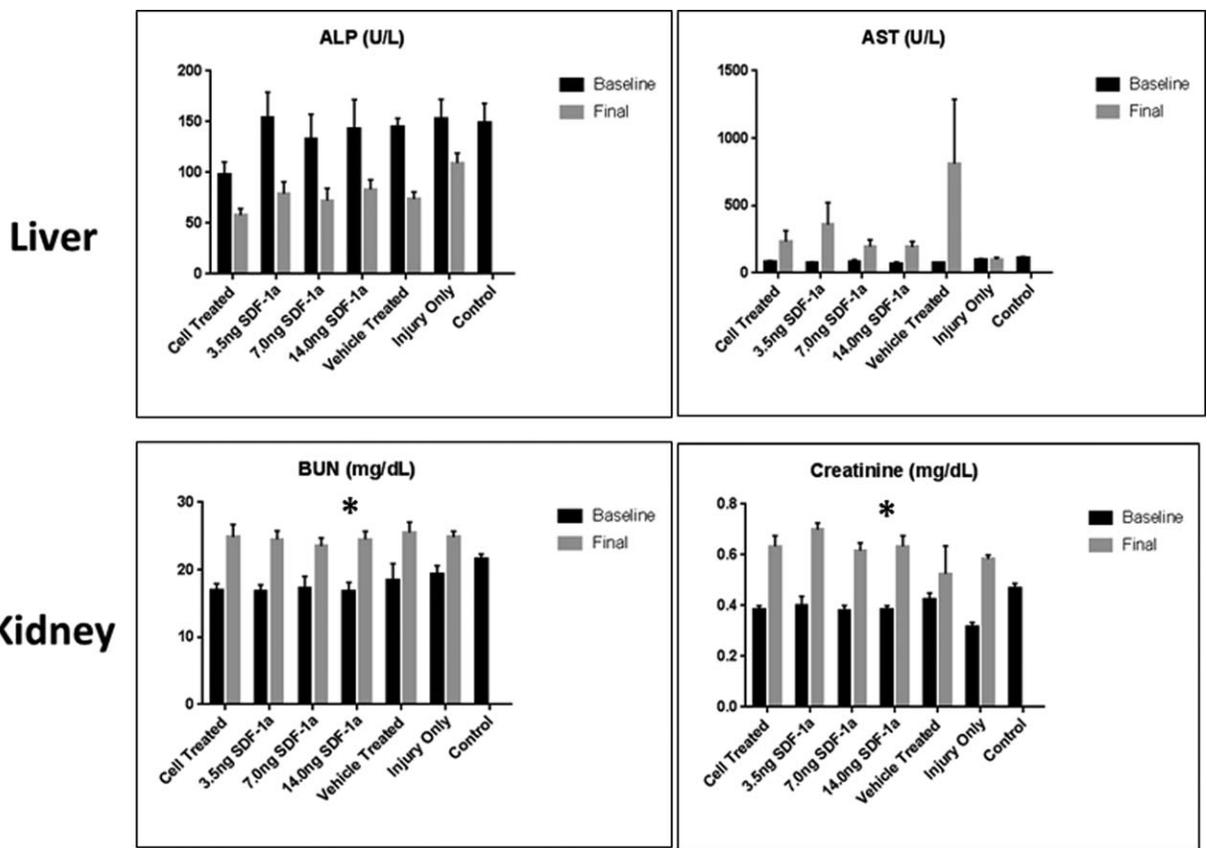


Figure 3. Liver and kidney function tests. Blood samples were taken at baseline (prior to any procedure) and then again just prior to necropsy (at the end of the treatment period). Values measured were, ALT and AST, BUN and creatinine. * $p < .05$ baseline versus post-treatment for the experimental groups of rats. Values are mean \pm SEM. Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen

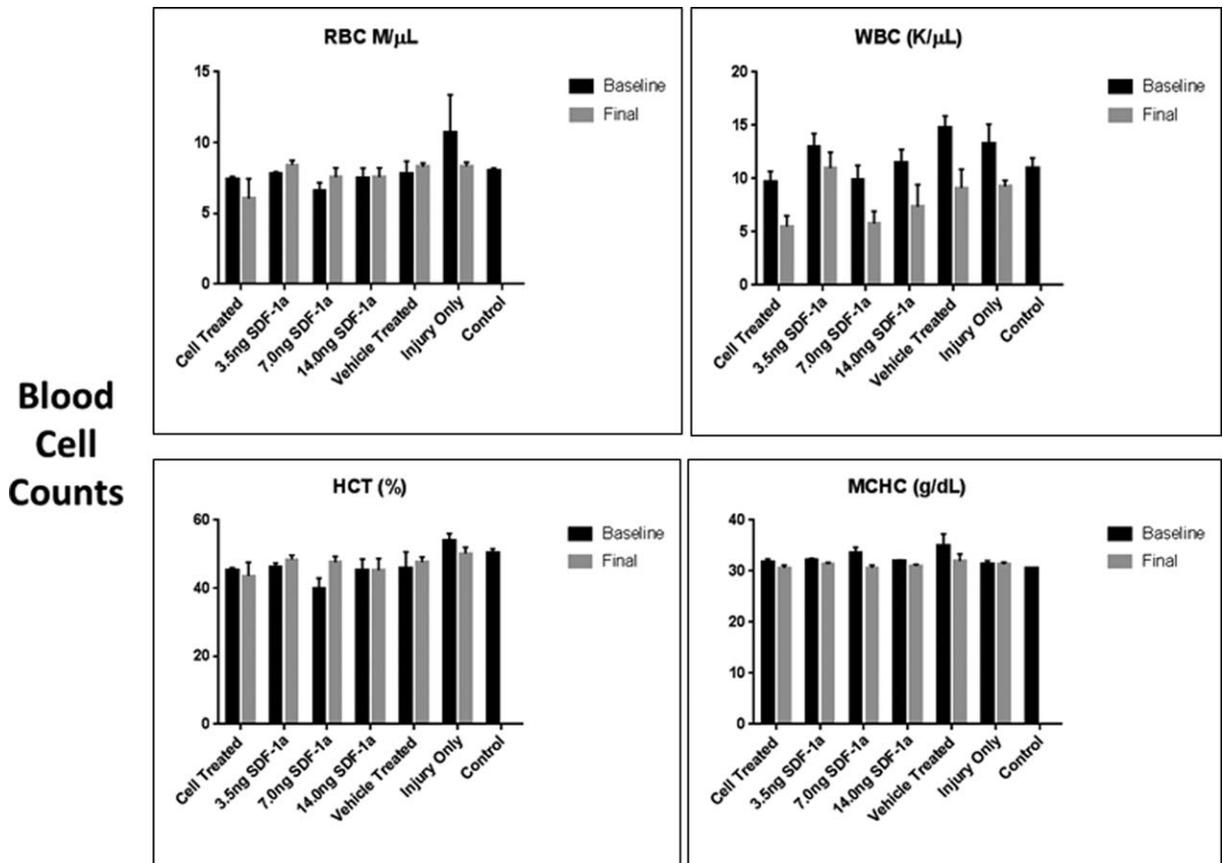


Figure 4. Red and white blood cell counts. This figure depicts the blood values of RBCs, WBCs, HCT and the MCHC in the experimental groups of rats. Abbreviations: RBCs, red blood cells; WBCs, white blood cells; HCT, hematocrit; MCHC, mean corpuscular hemoglobin concentrations.

studies focus on muscle damage [11, 16, 17]. The CXCL12 dose of 7 ng was calculated to be roughly equivalent to the 100 ng dose shown to be effective in nonhuman primates [11]. Half that dose (3.5 ng) was not as effective and twice that dose (14 ng) was no more effective. Therefore, we feel we are in the effective dose range of CXCL12.

Both skMPC and CXCL12 injections were effective in restoring control-like sphincter muscle and collagen content. It is unclear why skMPC injections would restore muscle content and not function. The innervation (as detected by PGP9.5) was restored in both treatment groups, theoretically, both should be functional. Chermansky et al report restoration of function and PGP9.5 expression in rats with experimental ISD. He injected 1.5 million and we injected 1 million cells. Perhaps this is a dose-effect of cells as we saw with the CXCL12. Mirroring the LPP results, the 7 ng dose of CXCL12 produced a maximum effect on the sphincter muscle content with less effect at a lower dose and no further effects of a larger dose.

The only consistent tissue pathology noted was some necrosis and hemorrhage in the urinary sphincter of the ISD/noTx rats. This confirms the injury protocol and shows that this pathology was not seen (except for a couple of cases) in the skMPC or CXCL12 treated rats. There was also bladder adhesions to the uterus noted in many of the animals. There was no other consistent pathology noted and no evidence of tumor growth. However, the treatment time was short (30 days) and may not have been sufficient to detect abnormal cells in the tissues. There is some concern about CXCL12 because of its vasculogenic properties and increased risk of cancer growth [14]. However, this was a one-time injection of CXCL12, which may reduce its risk of abnormal tissue response.

The blood work did not reveal any treatment effects of liver function, kidney function or red and white blood cell counts. However, the liver and kidney function measures were higher in all groups at necropsy (30 days post treatment). This was even true in the non-treated groups. There was no obvious reason for this unless the bladder adhesions placed some stress on the kidneys and the liver.

There are limitations in this study. As mentioned, LPP was measured in anesthetized rats, which may have affected the results. The treatment was given at the time of ISD induction and

not when chronic sphincter deficiency had been established. Furthermore, the treatment time was relatively short (30 days) and it is unclear whether longer treatment times would have affected the efficacy and/or the safety measures; especially the tumorigenic risk. We were not able to obtain consistent urine samples from the rats, which may have identified additional urologic pathology.

CONCLUSION

This study demonstrates a dose-response of CXCL12 therapy for the treatment of ISD in a rat model, and also provides an initial assessment of CXCL12 safety. Since efficacy was confirmed and no initial overt pathology was noted in these rats, this chemokine approach may represent a novel therapeutic option that bypasses the lengthy, expensive and less effective use of cell therapy for ISD. However, more extensive dose-responses, combined with longer-term safety studies will better assess its potential clinical utility.

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AUTHOR CONTRIBUTIONS

J.K.W.: Conception and design, financial support, collection and assembly of data, data analysis, manuscript writing, approval of manuscript; A.D.: Conception and design, collection and assembly of data, data analysis; S.L.: Conception and design, collection and assembly of data, surgery; K.-E.A.: Conception and design, manuscript writing, final approval.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

Dr. Williams wishes to disclose this patent application. U.S. Patent Application No. 15/309,093. *National phase of PCT/US2015/028490*. Inventor: James K. Williams. Title: *Methods of Treating Incontinence and Other Sphincter Deficiency Disorders*. Your Ref. 14-904; MB Ref. 9865-175.

The other authors indicated no potential conflicts of interest.

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