

Urodynamic Parameters in Spinal Cord Injury-Induced Neurogenic Bladder Rats after Stem Cell Transplantation: A Narrative Review

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What's Known

- Animal models of spinal cord injury, especially in rats, are the most commonly used method to evaluate the efficacy of cell therapy in neurogenic bladder improvement and the most common assessment method is urodynamic studies.
- There are variations in the range of urodynamic parameters among the published studies.

What's New

- Contusion injury is the most common spinal cord injury model. The average cell dose is about 1 million cells, transplanted 9 days after injury.
- In some urodynamic test results, to some extent, improvement in the function of lower urinary system is observed. However, this improvement is far from full functional recovery.

Abstract

Neurogenic bladder (NGB) secondary to spinal cord injury (SCI) is accompanied with several complications such as urinary tract deterioration, urinary incontinence, and consequently lower quality of life (QoL), significant morbidities, and occasionally death. Current therapeutic methods have some side effects and there is no treatment for the upper urinary tract injuries. Stem cell therapy is a promising method for treating this condition. However, the best timing and the best route of its transplantation have not yet been determined. Animal models of SCI, especially in rats, are the most commonly used method for evaluating the efficacy of cell therapy in NGB improvement, and the most common assessment method is the urodynamic studies (UDS). However, there are variations in the range of UDS parameters among the published studies. The current review aimed to discuss the effect of stem cell transplantation on bladder dysfunction recovery based on urodynamic parameters after SCI in rats. For this purpose, the cell source, doses, the route of administration, the complete UDS equipment and its parameters were summarized in SCI models in rats. In some urodynamic test results, to some extent, an improvement in the lower urinary system function was observed in each treatment group. However, this improvement was far from full functional recovery. The average cell dose was about 1 million cells in every injected site. In most studies, the stem cells (SCs) were transplanted 9 days after the injury using PE-50 and PE-60. Many researchers have recommended further experimental and clinical studies to confirm this treatment modality.

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Keywords • Urodynamic • Spinal cord injuries • Urinary bladder • Neurogenic • Stem cell transplantation

Introduction

NGB is a common condition in most neurological diseases such as SCI,¹ Parkinson's disease,² multiple sclerosis,³ and cerebral vascular accident/stroke⁴ which have a negative impact on QoL.⁵ The International Continence Society (ICS) has defined overactive bladder (OAB) as urgency (with or without urge incontinence), daytime frequency, and nocturia. In the presence of a neurological condition, this problem is called neurogenic OAB.⁶ Urine stasis and upper urinary tract injury following NGB

arises from high detrusor pressure during the filling phase (poor compliance) and additional contraction of the detrusor against the closed sphincter (detrusor sphincter dyssynergia).^{7,8} The prevalence of NGB in patients with SCI is 70% to 84%.⁹ NGB secondary to SCI is accompanied with several complications such as urinary tract deterioration,^{10, 11} urinary incontinence¹² and consequently lower QoL,¹³ significant morbidities and occasionally death.¹⁴ Numerous therapies for NGB including physical and psychological methods,¹⁵ electrical stimulation,¹⁶ medication,¹⁷ and surgery¹⁸ have been developed. However, these methods have some side effects and sometimes resulted in incomplete recovery.⁸

Stem cell transplantation and tissue engineering are the two important options that may overcome the limitations of the current therapies.¹⁹⁻²¹ Cell therapy is a sub-type of regenerative medicine. In this process, SCs are introduced into the tissue to treat a disease with or without gene therapy.²² The main SCs used for cell therapy are multipotent mesenchymal stem cells (MSCs)^{23, 24} with two important abilities, namely self-renewing and differentiation into various cell types.²⁵ MSCs are derived from bone marrow (BM-MSCs),²⁶ skeletal muscle (Sk-MSCs),²⁷ umbilical cord (UC-MSCs),²⁸ and adipose tissue.^{29, 30} Another source is adult tissue such as oral mucosa.³¹ For bladder regeneration, MSCs showed better results in comparison with using differentiated SCs.³² While numerous reports have shown the potential of these cells to replace or transplant in the lungs, liver, heart, and brain; however, data on improved bladder function is scarce.³³⁻³⁵ Some researches focused on the role of neural progenitor SCs, as multipotent adult SCs, that exist in the central nervous system in the regeneration of animal models.³⁶ The self-renewal potency and formation of new neurons and glial cells led researchers to use these cells.³⁷

Bladder substitutes, using synthetic or natural materials, have been developed in recent years. However, despite numerous attempts, it is still an immature process because of mechanical, structural, functional, or biocompatibility problems. Bladder acellular matrix (BAM)-based scaffold obtained from donor's bladders may overcome these problems. In this process, the cell and its components are removed from the bladder and then the tissue matrix with collagen, elastin, fibronectin, glycosaminoglycans, proteoglycans, and growth factors contents are placed.³⁸

The current review aimed to discuss the effect of stem cell transplantation on bladder dysfunction recovery based on urodynamic parameters after SCI in rats. For this purpose, the cell source,

doses, the route of administration, and the complete UDS equipment and its parameters were summarized in SCI models in rats.

Diagnosis of NGB

UDS is an important factor in the diagnosis of urethral and bladder dysfunction.³⁹ According to the definition of ICS, UDS is the study of lower urinary tract physiology and the urinary hydrodynamic transmission.⁶ Rodents, most common rats, are widely used to show urinary storage and voiding function both in normal conditions and in disease models⁴⁰ using cystometry as a common methodology. Cystometry in rodents gives important information about the physiology and pharmacology of the bladder.⁴¹

Cystometry in Rodents

There are morphological and functional differences between the bladder of humans and rats.⁴¹ In rats, bladder contraction is mediated by ATP, whereas in humans it is contracted by the mediation of acetylcholine.⁴⁰⁻⁴² Cystometric parameters in rodents are poorly defined and the used criteria are different from humans. Therefore, it is important to define the terminology on what is measured and avoid using the terminology for human cystometry.⁴¹

The reviewed articles had considered two different methods for urodynamic testing in rats. The first method was performed by using a cystometry catheter (PE-50) "Y" connected to a continuous infusion system and to a polygraph. In this method, vesical pressure during the filling and voiding phase is determined and the maximum urethral closure pressure is determined with an indirect method.⁴⁰ The second method is performed using a catheter (external diameter of 0.64 mm and internal diameter of 0.5 mm) with two orifices inserted via the urethra to the bladder and "Y" connected to the infusion pump and polygraph. In this method, the behavior of the bladder during urination is determined.⁴³

Anderson reported on a rodent UDS model⁴⁰ and noted that a control or sham group should be present in every cystometric study.⁴⁰ The common parameters that are usually reported in UDS include pressure parameters such as the baseline pressure or minimum pressure between two micturitions (usually between 10 to 20 cmH₂O), intermicturition pressure or mean pressure between two micturitions (related to the occurrence of non-voiding contractions), threshold pressure or intravesical pressure (immediately before micturition, maximum pressure during a micturition cycle or peak pressure, maximum voiding pressure, or

maximum intravesical pressure), micturition frequency (the number of micturitions per hour), bladder capacity (determined as infused volume divided by the number of micturitions, provided there is no residual volume), and ICI (defined as the time period between two maximum voiding pressures). Volume parameters are the other cystometric parameters in the evaluation of the rodent bladder. It includes micturition volume (volume after a micturition minus volume before a micturition) and residual volume (the volume remaining in the bladder after voiding). Another important parameter of rodent UDS is bladder compliance (bladder capacity divided by threshold minus basal pressure).⁴⁰

Models of SCI to Induce NGB

The contusion model of SCI is reported in some articles at the T10-T12 vertebral level,⁴⁴ T8-9 level of the spinal cord,²³ at mid-thoracic level of T9,⁴⁵ at the 10th thoracic level of the spinal cord segment,⁴⁶ with the NYU impactor (10 g, 25 mm)⁴⁷ injury at the T9 vertebral level of the spinal cord⁴⁸ or laminectomy of the T10 spinal vertebrae, and complete transection at the lower thoracic level of the spinal cord.⁴⁹ Temeltas and others performed a laminectomy, following anesthesia, at the T9-T10 level and induced a hemisection SCI model via dura incision above the dorsal root entry zone and cutting with micro-scissors at the rostral and caudal extents of the injury. Then, aspiration was done to ablate only the lateral white matter tracts and a minimal portion of the dorsal and ventral gray matter.⁵⁰

The contusion injury model is the most common SCI model. In this model, the dorsal region of the spinal cord is damaged or destroyed. Rostral to the lumbosacral level of SCI leads to bladder hyperreflexia, detrusor sphincter dyssynergia, and finally bladder hypertrophy.⁴⁹ Complete deterioration of bladder compliance, function, infection, and lower urinary tract complications are the other sequels of SCI.⁵¹ In these studies, the mechanism of bladder dysfunction induced by SCI reported overactivity.

The Timing of SCs Transplantation After Injury

Different studies used different timing for the transplantation of SCs. It ranged from immediate transplantation of cells after SCI⁴⁴ to 8 weeks after SCI.⁴⁹ However, in most studies, the SCs were transplanted 9 days after injury. Cho and others⁴⁴ infused 100 μ l of oral mucosa SCs immediately after SCI. Park and others²³ used the timing of 9 days after injury in which human MSCs were placed in the contusion site of the spinal cord. Sandner⁴⁵ injected bone

marrow stromal cells 3 days after inducing SCI in the center of the lesion. Obara⁴⁶ grafted BAM 2-3 weeks after SCI in rats that had undergone partial cystectomy 13 weeks after contusion. Jin⁴⁷ transplanted neural progenitor cells by using a 10- μ l NanoFil™ syringe (World Precision Instruments, Sarasota, FL, USA) with a 33-gauge needle. In their process, the dura in the center of the lesion did not open and remained for 1 minute after injection.

In a study conducted by Lee,⁴⁸ human bone marrow MSCs labeled with magnetic nanoparticles were transplanted into six areas of the bladder muscle of rats 4 weeks after the SCI operation. Urakami⁴⁹ performed bladder replacement surgery 8 weeks post-injury. The urinary bladder was exposed via a small suprapubic incision and partial cystectomy (50%), then BAM graft was anastomosed to the host bladder with running and interlocking 7-0 absorbable sutures. Temeltas and others⁵⁰ implanted a mixed population of neuronal restricted precursors (NRP)/glial restricted precursors (GRP) or culture medium transplantation, into the injury cavity 9 days after hemisection.

The best route of administration and site of transplantation for different diseases and the possible contraindication of clinical usage are still unknown.³² Also, the time of cell transplantation is very important⁵² for the therapeutic effect.³² The characteristics of the reviewed studies are described in table 1.

The Methods and Time of Catheterization to Perform Cystometry after SCI

In a study by Cho and others,⁴⁴ after anesthesia, a sterile polyethylene catheter (PE50) was inserted into the bladder connected to a pressure transducer (Harvard Apparatus Inc., Massachusetts, USA) to record the intravesical pressure as well as a syringe pump to infuse saline into the bladder. After emptying the bladder, cystometry was performed. Park and others²³ conducted cystometry at 28 days and 56 days after transplantation. Sandner⁴⁵ catheterized the bladder 3-4 days prior to urodynamic measurements. After anesthesia, the bladder was exposed by a midline incision in the low abdominal wall and a PE catheter with a cuff was inserted into the bladder dome and then secured by a purse-string suture and externalized through the skin in the back of the neck. The external tip of the catheter was closed thermally, to prevent leakage and infection, and sutured to the skin until the urodynamic measurements. Urodynamic measurements were performed 8.5 weeks post-injury in conscious rats.

Table 1: Characteristic of SCI and stem cell types in the reviewed studies

| Reference | SCI type | Device name for injury | The number of rodents in each group | Cell type for transplantation | Cell labeling | Time of transplantation after SCI | Route of transplantation | Cell count | Injured vertebrate segment |
|------------------------------|---------------------|--|-------------------------------------|-------------------------------|------------------|---|--|----------------------------|----------------------------|
| Cho⁴⁴ | Needle-stick injury | 22-gauge needle | 10 | Oral mucosa stem cells | | Just after SCI | Stem cells were infused over the course of 1 min by using a 22-gauge insert vein (IV) catheter intrathecal | 100 μ l | T11 |
| Park²³ | Contusion | Multicenter animal SCI study (MASIS) impactor (Rutgers, The State University of New Jersey, Newark, NJ), a 10-gram rod was dropped from a vertical distance of 25 mm | 6-11 | hMSCs | | 9 days after injury | Into the injured spinal cord via Hamilton syringe | 5 μ L | T9 |
| Obara⁴⁶ | Compression | Compression with a 40-gram rod (tip area: 3.0-2.2 mm) placed on the exposed dura for 30 minutes | 5 and 15 | BAM grafting | | 2 to 3 weeks after BAM grafting | A BAM was firmly anastomosed to the host bladder with running and interlocking 7-0 absorbable sutures. | | T10 |
| Sander⁴⁵ | Contusion | Infinite Horizon (IH) impactor SCI device (Precision Systems and Instrumentation, Lexington, KY, USA) with an impact force of 200 kilodynes (kdyn) | 6-8 | BMSCs | | 3 days postinjury | Into the center of the lesion at a maximum depth of 1.5 mm using a pulled glass pipette (100 μ m internal diameter) and a Picospritzer II | 1 \times 10 ⁵ | T9 |
| Urakami⁴⁹ | Transection | Micro scissors | | BAM grafting | | 8 weeks after SCI (bladder replacement) | The BAMG was anastomosed to the host bladder with running and interlocking 7-0 absorbable sutures | | T10 |
| Temeltas⁵⁰ | Hemisection | Micro scissors | 6-10 | NRP/GRP | | 9 days after hemisection | Into the injury cavity | 1 \times 10 ⁶ | T9-T10 |
| JIN⁴⁷ | Contusion | NYU impactor (10 g, 25 mm) | 9-10 | NPCs | | 13 weeks after SCI | Using a 10- μ l NanoFil syringe with a 33-gauge needle, 1 \times 10 ⁵ cell/ μ l were injected into the lesion center along the midline (4 μ l) and rostral and caudal to the lesion along the midline (3 μ l/each). | | T10 |
| Lee⁴⁸ | Contusion | Chung-Ang University Hospital Model 2.0 (CAUH-2) pneumatic impactor (3 mm depth) | 10 | BM3.B10 | Fluorescent MNPs | 4 weeks after SCI | Into the bladder muscle of the rats in six areas | 1 \times 10 ⁶ | T9 |

BAM: Bladder acellular matrix; hMSCs: Human mesenchymal stem cells; BMSCs: Bone marrow stromal cells; NRP: Neuronal restricted precursors; GRP: Glial restricted precursors

In a study by Obara,⁴⁶ cystometry was performed at 5, 7, and 15 weeks in the intact-BAM and SCI-BAM rats. A polyethylene catheter was placed via the urethra into the bladder and was connected to the pressure transducers and then the pressure signals were recorded. Jin⁴⁷ performed cystometry in conscious rats 8 weeks after transplantation (22 weeks after initial injury) by using a polyethylene catheter. After catheterization, the rats recovered for 1-2 hours and were then placed in a restraining cage (KN-326, Natsume, Tokyo, Japan). A pressure transducer (Bladder pressure transducer, Germany) and infusion pump (STC-523, Terumo, Tokyo, Japan) were used in this procedure. In a study by Lee,⁴⁸ voiding response was assessed at 4 weeks after transplantation. After anesthesia, the bladder was exposed via a midline abdominal incision and a catheter was inserted through a small incision in the bladder dome. After passing the other end of the catheter subcutaneously, its end was exited through the skin. Urakami⁴⁹ performed cystometry before grafting and repeated 8 weeks after bladder replacement. After anesthesia, cystometry was performed after emptying the bladder. In a study by Temeltas and others,⁵⁰ UDS was conducted 4 weeks after transplantation similar to the previously mentioned method.

The time of catheterization to perform UDS varied among different studies. However, the timing was in the range of 2 to 8 weeks after transplantation. The catheter was inserted via the urethra or the bladder, usually in the dome, secured by a purse-string suture, tunneled subcutaneously, and externalized through the skin at the back of the neck. In these studies, PE-50 and PE-60 were used. However, in other UDS in rats, PE-10,⁵³ PE-50,⁵⁴⁻⁵⁷ PE-60,⁵⁸ PE-90,^{59, 60} and PE-100⁶¹ were used.

The Rate of Infusion into the Bladder to Induce Micturition to Perform Cystometry and Measure Parameters

Cho and others⁴⁴ infused 0.5 ml normal saline and conducted a cystometry. Park and others²³ infused 10 ml/hour normal saline at room temperature into the bladder. Then, the rate was reduced to 5 ml/hour. For stabilizing the micturition cycles, after the first void, the infusion was stopped every 30 minutes. Detrusor pressure, timing, and frequency of voiding were recorded. It should be noted that the cystometry in their study was conducted under the anesthetic vapor with low level (0.5%) to reduce the effect of anesthesia on the micturition cycle.

In a study by Sander,⁴⁵ to measure repeated

micturition cycles, the bladder was filled via the implanted catheter with saline for 30-45 minutes at a rate of 6 ml/h. During UDS, the bladder pressure and urine volume were recorded. To measure micturition volume, released fluid was collected through a funnel into a cup connected to a force transducer. For pressure measurements, the bladder catheter was connected via a three-way stopcock to a pressure transducer and to a syringe pump. Both transducers were connected to an amplifier and a data acquisition system. The measured parameters were micturition volume (MV), micturition frequency (MF per hour), basal pressure (P_{base} : minimum pressure between two micturition events), maximum voiding pressure (P_{max} : maximum bladder pressure during a micturition cycle), and pressure difference (P_{diff} : $P_{max} - P_{base}$).

In a study by Obara,⁴⁶ saline 0.9% at room temperature was infused into the bladder continuously at a constant rate of 0.1ml/min. They only checked the leak-point volume and pressure when saline leaked from the meatus around the tube in anesthetic rats. Jin⁴⁷ performed UDS by infusion saline with a rate of 0.1 ml/min. Lee⁴⁵ recorded UDS by infusion of physiological saline at room temperature into the bladder at a rate of 0.04 ml/min. Urakami⁴⁹ performed a cystometry with saline infusion into the bladder at the rate of 0.2 ml/min. The measured parameters were bladder pressure during the filling phase, bladder capacity, threshold voiding pressure, bladder compliance (by dividing the bladder capacity by the threshold voiding pressure), voided volume, post-void residual volume, uninhibited detrusor contractions (UIC), maximal amplitude of UIC, and infused saline volume at the first UIC (table 2).

Temeltas and others,⁵⁰ 4-6 hours after recovery from anesthesia, infused saline at room temperature at the rate of 0.2 ml per minute and monitored intravesical pressure. The saline infusion was halted during the micturition process. The voiding saline from the rat's urethral meatus was measured to a designated voided volume and post-void residual urine volume was then measured. The residual saline amount was withdrawn via an intravesical catheter and the bladder of the rats was squeezed manually on the abdominal wall and the remaining intravesical amount collected. Voided volume and post-void residual volume calculated as bladder capacity. Different infusion rates have been described for rat urodynamics from 2.4 ml/hr to 11 ml/hr.^{40, 53-62} In these studies, the rate of saline infusion was in the range of 0.04 ml/min to 0.1 ml/min or 6 ml/h. The characteristics of urodynamics of the studies are described in table 2.

Table 2: Characteristic of UDS in the reviewed studies

| Reference | The time of cystometry after bladder catheterization | Time of catheterization to perform cystometry after SCI | The type of catheter used for cystometry | Animals status in cystometry (conscious or anesthetized) | The type of anesthetic drug for catheterization | Cystometry set-up | The rate of infusion into bladder |
|------------------------|--|---|--|--|---|---|-------------------------------------|
| Cho ⁴⁴ | Just before cystometry | 21 days after SCI | PE-50 | Anesthetized | Zoletil | Pressure transducer and a syringe pump (Harvard Apparatus, Holliston, MA, USA) monitored using LabScribe (iWork System, Inc., Dover, NH, USA). | 0.50 ml/min |
| Park ²³ | Just before cystometry | 28 and 56 days after injury | Double lumen polyethylene catheter (PE-160 and PE-50; Clay Adams, Parsippany, NJ, USA) | anesthetized | Isoflurane | Pressure transducer by polygraph (Grass polygraph model 7E, Quincy, MA, USA) and infusion pump (Baxter, Deerfield, IL, USA). | First 10 ml/hour and then 5 ml/hour |
| Obara ⁴⁶ | Just before cystometry | 5, 7, and 15 weeks after SCI | PE-60 (Clay Adams, Parsippany, NJ, USA) | Anesthetized | Pentobarbital sodium | Pressure transducers (Statham P-23, Gulton-Statham Transducers, CA, USA) and recorded by (Menuet Compact, Dantec Medical A/S, Denmark). | 0.10 ml/min |
| Sander ⁴⁵ | 3 to 4 days prior to urodynamic measurements | 8.5 weeks post injury | PE catheter (diameter 2.1 mm, PE-50 (Schubert Medizinprodukte, Wackersdorf, Germany) | Conscious | Ketamine, xylazine, and acepromazine | Force transducer (MLT1030/D wide range force transducer; AD Instruments, Oxford, UK), pressure transducer (MLT0699 disposable BP transducer; AD Instruments, New Zealand) and to a syringe pump (KR Analytical, Cheshire, UK), data acquisition system (PowerLab 8/35; AD Instruments, New Zealand) | 6 ml/h |
| Urakami ⁴⁹ | Just before cystometry | Before grafting, 8 and 16 weeks after SCI and bladder replacement | 22-gauge catheter | Anesthetized | Ketamine | | 0.20 ml/min |
| Temeltas ⁵⁰ | 4 to 6 hours after recovery | 4 weeks after transplantation | PE-50 | | ketamine+xylazine | Biopac Systems™ device | 0.20 ml/min |
| JIN ⁴⁷ | 1-2 hour before urodynamic measurements | 22 weeks after SCI and 8 weeks after transplantation | PE-60 (Clay Adams, Parsippany, NJ, USA) | Conscious | Isoflurane | Pressure transducer (BLPR; World Precision Instruments) (BLPR; Bladder pressure transducer, Germany) and infusion pump (STC-523; Terumo, Tokyo, Japan) | 0.10 ml/min |
| Lee ⁴⁸ | 5-6 hour before urodynamic measurements | 4 weeks after cell transplantation and 8 weeks after injury | PE-50 (Harvard Clinical Technology, Inc., South Natick, MA, USA) | Conscious | Isoflurane | | 0.04 ml/min |

PE: Polyethylene tube

The Results of UDS

Cho⁴⁴ determined the contraction pressure 2.55 ± 0.22 cmH₂O in the sham-operation group, 6.49 ± 0.77 cmH₂O in the SCI-induced group, and 3.05 ± 0.14 cmH₂O in the SCI-induced and oral mucosa stem cell transplantation group. The results showed that bladder contraction pressure and contraction time were increased in the SCI group, whereas they were improved after the transplantation of oral mucosa SCs.

Park and others²³ showed that the voiding frequency (time/min) was 0.80 ± 0.09 and 0.82 ± 0.16 in two control groups and 0.79 ± 0.11 in an experimental group at 28 days after injury. Sandner⁴⁵ observed a similar baseline bladder pressure, a slightly increased maximal voiding pressure, and a significantly increased pressure difference. In addition, spinal cord-injured animals frequently showed non-voiding contractions, which was not observed in naive animals. In treated animals, a tendency toward a decrease of the micturition frequency was reported compared to non-injected injured rats; although this difference was not statistically significant (ANOVA $P=0.0535$ comparing all injured groups). The voided volume per micturition of all injured animals was decreased compared to intact animals but was not significant (ANOVA $P=0.06$). In both fibroblast-grafted and BMSC grafted animals compared to the injured control animals, the voided volume per micturition was slightly high and the difference among the injured group and control group was not statistically significant (ANOVA $P=0.28$).

In a study by Obara,⁴⁶ due to the pentobarbital anesthesia effect on suppressing the neural circuit of the micturition reflex, the intravesical pressure rose gradually until voiding was initiated. The leak point volume of the SCI rats was significantly higher than of the intact rats, and the leak-point pressure of the SCI rats was significantly lower than of the intact rats.

Jin⁴⁷ observed bladder deficiency in all experimental groups in comparison with the intact group. As shown in table 3, these values increased in all experimental groups compared to the intact group ($P < 0.05$). Non-voiding contractions in the intact group was zero, while in other groups were 7.4 ± 3.1 , 6.0 ± 0.6 , 7.0 ± 1.2 and 7.8 ± 1.5 , respectively. The voided volume (ml) ranged between 0.3 in the intact group to 4.13 in the group of NPCs+lentivirus vectors expressing chondroitinase and neurotrophic factors (N/C/G group). The bladder contraction duration and amplitude of micturition were also high in three of the four experimental groups compared to the intact animals, except for the N/C/G group which was similar to normal values.

After combined treatment, bladder function improvement was observed. The interaction intervals (sec) were 148.9 ± 10.5 in the intact group, 214 ± 40.9 in medium (control) group, 289.8 ± 78.8 in neural progenitor cells transplants (NPCs) group, 258.2 ± 38.4 in NPC+lentivirus vector expressing chondroitinase group, and 230.5 ± 69.2 in NPC+lentivirus vectors expressing chondroitinase and neurotrophic factors. Bladder contraction duration (sec) was 19.1 ± 2.5 , 28.6 ± 3.9 , 28.2 ± 6.2 , 31.8 ± 4.6 , and 18.9 ± 3.5 , respectively. The amplitude of micturition (cmH₂O) was 35.0 ± 6.3 , 44.3 ± 7.8 , 42.3 ± 6.3 , 47.9 ± 7.2 , and 34.7 ± 2.5 , respectively. Residual volume (ml) was zero in the intact group, 0.177 in medium group, 0.1 in NPC group, 0.05 in N/C, and 0.05 in N/C/G group.

Lee and others⁴⁸ showed that following SCI, the inter-contraction interval (ICI: the interval between voids or reflex bladder contractions) was low. However, after cell transplantation, the inter-contraction interval was increased. According to their figures, the ICI (sec) was about 340 in normal rats, 370 in the sham group, 50 in the SCI group, and about 160 in the SCI+B10 cell group. The pressure threshold (PT) was similar between the groups. The amount of PT (cmH₂O) in the control group was about 8, 9 in sham group, 7 in the SCI group, and about 9 in the SCI+B10 cell group. After SCI, the amount of maximum voiding pressure was increased, but it decreased after B10 cell transplantation.

In a study by Urakami,⁴⁹ 8 weeks after spinalization, 22 rats (71.0%) had hyperreflexic bladders with UIC and 9 rats (29.0%) had underactive bladders with no UIC. The mean bladder capacity and RUV of SCI rats (4.2 ± 2.3 , 1.5 ± 1.6 ml, respectively) were remarkably higher than normal rats (0.5 ± 0.1 , 0.05 ± 0.02 ml, respectively). Similar urodynamic results were seen in SCI-control rats (with no bladder replacement) 8 weeks and 16 weeks after spinalization. No uninhibited contraction was observed in all ten normal rats. The bladder capacity was 1.0 ml and the maximum amplitude of the voiding contractions was 31.0 cm/H₂O.

A typical cystometrogram in spinalized rat showed many and large uninhibited non-voiding contractions (more than 15 cm/H₂O) during saline infusion. The size of the contractions increased with time. Bladder capacity was approximately 2.5 ml, threshold pressure was 33.5 cm/H₂O, and bladder compliance was 0.075. No uninhibited contractions during saline infusion were seen in a typical cystometrogram of SCI-induced underactive-bladder (areflexia). The bladder capacity exceeded 4.0 ml and bladder compliance was very high.

Table 3: Measured urodynamic parameters in SCI-induced neurogenic bladder in the reviewed studies

| Reference | Groups | Contraction pressure (cmH2O) (mean±SD) | Contraction time (sec) (mean±SD) | Voiding pressure (mean±SD) | Voided volume (mean±SD) | Voiding frequency (time/min) (mean±SD) | Maximal pressure (mean±SD) | Voiding pressure (VP) (mean±SD) | Pressure threshold (PT) (mean±SD) | Bladder capacity (mean±SD) | Bladder compliance (mean±SD) | Post-void residual volume (mean±SD) | Baseline pressure (cmH2O) (mean±SD) |
|------------------------|---|--|----------------------------------|----------------------------|-------------------------|--|----------------------------|---------------------------------|-----------------------------------|----------------------------|------------------------------|-------------------------------------|-------------------------------------|
| Cho ⁴⁴ | Control | 2.55±0.22 | 8.09±0.43 | | | | | | | | | | |
| | Negative control | 6.49±0.77 | 15.29±1.65 | | | | | | | | | | |
| Paik ²³ | Experimental | *3.05±0.14 | *13.36±0.40 | | | | | | | | | | |
| | Control 1 | | | 0.80±0.09 | 9.21±5.30 | | | | | 1345.25±1206.27 | | | |
| | PBS (n=7) | | | | | | | | | mm ³ | | | |
| Lee ⁴⁸ | Control 2 | | | 0.82±0.16 | 9.60±4.20 | | | | | 1263.75±638.05 | | | |
| | hFb (n=11) | | | | | | | | | mm ³ | | | |
| | Experimental | | | 0.76±0.32 | 9.70±4.11 | | | | | 1279.57±1310.69 | | | |
| *Sander ⁴⁵ | hMSC (n=9) | | | | | | | | | mm ³ | | | |
| | Control | | | | | | 39.24±2.64 | | 8.18±0.77 | | | | |
| | Sham | | | | | | 43.77±1.50 | | 8.50±0.54 | | | | |
| | SCI | | | | | | 77.73±2.64 | | 7.13±0.72 | | | | |
| | SCI+B10 | | | | | | 57.35±4.15* | | 8.54±0.31* | | | | |
| Urakam ⁴⁹ | SCI | | | 23.00±2.25 | 72.60±9.56 | | | | | | | | 26.38±6.38 |
| | BMSC-grafted | | | 19.75±1.87 | 81.73±9.13 | | | | | | | | 29.36±3.82 |
| | Fibroblast-grafted | | | 15.50±1.12* | 65.65±6.52* | | | | | | | | 19.57±2.55* |
| Temeltas ⁵⁰ | Control | | | | | | | | | 0.50±0.10 | | | |
| | Before graft | | | | | | | | | | 0.37±0.07 | 1.38±0.20 | |
| | After BAM-grafted | | | | | | | | | | | *P<0.0001 | |
| JIN ⁴⁷ | Sham | | | 24.90±4.03 | 0.56 | | | 24.90 | | 0.57±0.13 | | 0.05±0.07 | 2.15±0.54 |
| | SCI+neural restricted precursor | | | *43.40±5.71 | 1.02 | | | *43.40 | | *1.64±0.13 | | *0.64±0.12 | 3.38±0.30 |
| | SCI+BM-SCs | | | *53.10±7.88 | 0.90 | | | *53.10 | | *1.70±0.10 | | *0.79±0.20 | *5.16±1.03 |
| Urakam ⁴⁹ | SCI control | | | 61.80±9.41 | 0.27 | | | 61.80 | | 1.89±0.18 | | 1.61±0.23 | 8.78±0.55 |
| | Intact | | | | 0.30 | | | | | 0.3±0.00 | | 0.00 | |
| | Medium (control) | | | | 3.63 | | | | | 3.8±0.90 | | 0.17 | |
| | NPC transplant | | | *5.20 | *4.05 | | | | | *5.3±1.20 | | *0.10 | |
| | NPC transplant with Chase/LV (N/C) | | | | | | | | | *4.1±0.80 | | *0.05 | |
| Urakam ⁴⁹ | NPC transplant with Chase/LV+BDNF/NT-3/LV (N/C/G) | | | | | | | | | | | *0.05 | |
| | NPC transplant with Chase/LV+BDNF/NT-3/LV (N/C/G) | | | | | | | | | | | *0.05 | |

*P<0.05: Improvement of bladder function after stem cell transplantation or grafting. **There was no quantitative information about the cystometric parameters in the text and the result was written based on figure information. BM-SCs: Bone marrow stromal cells; NPCs: Neural progenitor cells; Chase/LV: Lentiviral vector expressing chondroitinase; Chase/LV+BDNF/NT-3/LV: Lentiviral vector expressing chondroitinase and growth factor treatments, B10 cell: BM3.B10 (B10); hMSCs: Human mesenchymal stem cell; PBS: Phosphate-buffered saline; hFbs: Human fibroblasts

Temeltas⁵⁰ reported that the mean±SD of baseline pressure (cmH₂O) in the sham operation group was 2.15±0.54, in the SCI group that received NRP/GRP it was 3.38±0.30, in the SCI group that received bone marrow stromal cell transplantation 5.16±0.55, and in the SCI control group 8.78±0.55. The mean±SD of maximum bladder capacity (ml) was 0.57±0.13, 1.64±0.13, 1.70±0.10, and 1.89±0.18, respectively. The frequency of uninhibited detrusor contractions per minute was in the range of 0.20±0.44 to 5.80±2.58 and the amplitude (cmH₂O) was in the range of 1.20±2.68 to 32.01±10.19. The mean±SD of voiding pressure (cmH₂O) was 24.90±4.03 in the sham operation group, 43.40±5.71 in group 2, 53.10±7.88 in group 3, and 61.80±9.41 in the SCI control group. The mean±SD of voided volume (ml) was 0.56±0.12 in group 1, 1.02±0.08 in group 2, 0.90±0.18 in group 3, and 0.27±0.05 in group 4. Post-void residual volume (ml) was in the range of 0.05±0.07 to 1.61±0.23. Urodynamic parameters were poor in all other groups compared to the sham operation group (P=0.0001). After treatment, the level of baseline pressure, maximum capacity, and amplitude of involuntary contraction during the filling phase were improved (P=0.0001, P=0.001, and P=0.001, respectively). The number of involuntary contractions was not significantly different between the treatment groups and group 4. Mean voiding pressure and post-void residual urine volume in the treatment groups were lower than the control group (P=0.0001 and P=0.001, respectively) while voiding volume was greater (P=0.001).

In the reviewed articles, several cystometric parameters such as cystometric pressure parameters (baseline, intermicturition, threshold, maximal, voiding and non-voiding pressure), micturition frequency, volume parameters (voided volume, post-void residual volume), and bladder compliance were measured. In some studies, only contraction pressure and time with a range of 2-6 cmH₂O and 8-15 second, respectively, were measured.⁴⁴ In another study, only maximal pressure was measured.²³ Three studies reported bladder capacity^{45, 49, 50} with a minimum range of 0.3 to a maximum range of 5.3 ml.^{44, 46, 47} Some important results of UDS in the above-mentioned studies are summarized in table 3.

Discussion

SCI-induced bladder dysfunction is one of the most debilitating functional deficits.^{63,64} Among different treatment methods, stem cell transplantation is promising. In some urodynamic

test results, to some extent, improvement in the function of the lower urinary system in each treatment group was observed. However, this improvement was far from full functional recovery. The remaining unknowns for the best therapeutic effect are the route of administration, cell doses, timing, the site of cell transplantation for different diseases, and possible contraindication of clinical usage.^{32, 46} Another concern in the field of stem cell therapy is the diminishing regenerative potential of aged SCs. Thus, the success of future clinical trials will depend on experimental investigations.⁶⁵ There were different timings for stem cell transplantation among the reviewed studies, ranging from immediate transplantation of cells after SCI⁴⁴ to several weeks after SCI.⁴⁹ The average cell dose was about 1 million cells in every injected site and, in most studies, the SCs were transplanted 9 days after injury. Transplanted cell survival is still another significant challenge that limits further recovery in SCI. In this regard, the main limitation is the high post-transplantation cell mortality.⁶⁶ Various strategies for improving the survival of SCs have been proposed.⁶⁷ However, none of the reviewed studies had any mention of such strategies.

To examine voiding functions, an implanted catheter into the bladder dome (by avoiding bladder outlet obstruction and providing the ability to perform urodynamic measurements in conscious rats) was preferred. Different infusion rates from 2.4 ml/hr to 11 ml/hr have been described for rat urodynamics. In the reviewed studies, the rate of saline infusion ranged from 0.04 ml/min to 0.1 ml/min or 6 ml/hr. PE-50 and PE-60 were also used; however, PE-10,⁵³ PE-50,⁵⁴⁻⁵⁷ PE-60,⁵⁸ PE-90,^{59, 60} and PE-100⁶¹ were used in other urodynamic studies in rats. It was assumed that the thinner tubes (PE 10) had a higher resistance.⁶⁸

Several cystometric parameters such as pressure parameters, volume parameters, and bladder compliance were measured. Some studies only measured the contraction pressure and time with a range of 2 to 6 cmH₂O and 8 to 15 second, respectively. In another study, only the maximal pressure was measured. Three studies reported bladder capacity with a minimum range of 0.3 to a maximum range of 5.3 ml. Also, conscious rats had different bladder behavior compared to anesthetized rats. In the present review, approximately 50% of the studies were conducted on conscious rats and some others used common drugs such as isoflurane and/or ketamine for anesthesia. Ozkurkcugil surveyed the effect of different anesthetic drugs on cystometric parameters in comparison with the conscious condition.

The effect of ether and midazolam on the parameters were similar in both conditions. However, propofol altered these parameters but was not statistically significant.⁶⁹

Despite the claim that ketamine has no effect on cystometric parameters, Ozkurkucugil reported that it had a high depressant effect on micturition. Therefore, it may be not suitable for experimental studies using urodynamic parameters in rats.⁶⁹ Isoflurane may be the best choice for small surgical procedures because it has a short half-life. However, in a study by Chang and others, isoflurane led to the prolongation of bladder inter-contractile intervals.⁷⁰ Hence, anesthetic properties should be taken into account in the experimental design and interpretation of urodynamic findings in rodent models.

Conclusion

In the present study, the effect of stem cell transplantation on bladder dysfunction recovery based on urodynamic parameters after SCI in rats was reviewed. For this purpose, the cell source, doses, route of administration, the complete UDS equipment and its parameters were summarized in SCI models in rodents. Among the reviewed studies, the contusion injury model was the most common SCI model. The average cell dose was about 1 million cells in every injected site. Also, the SCs were transplanted into the lesion cavity 9 days after injury.

In some urodynamic test results, to some extent, an improvement in the function of the lower urinary system in each treatment group was observed. However, this improvement was far from full functional recovery. Since we did not assess its effect on the other neurological conditions, it cannot be generalized to the other neurological diseases. Hence, it is recommended to conduct a comprehensive review of all neurological conditions that lead to NGB and to compare urodynamic parameters between treated and control groups.

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