ORIGINAL ARTICLE

Improvement in detrusor-sphincter dyssynergia by bladder-wall injection of replication-defective herpes simplex virus vector-mediated gene delivery of kynurenine aminotransferase II in spinal cord injury rats

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Study Design: An experimental study.

Objectives: To investigate the effect of replication-defective herpes simplex virus (HSV) vectors encoding the kynurenine aminotransferase II (HSVrd-KATII) gene on detrusor-sphincter dyssynergia (DSD) in spinal cord injury (SCI) rats.

Setting: Beijing, China.

Methods: Sprague–Dawley rats (240–265 g) were spinalized with complete transaction at the T10 level of the spinal cord. The rats were randomly divided into the following three groups: sham group ($n = 12$, with normal saline); HSVrd group ($n = 12$, with HSVrd) and HSVrd-KATII group ($n = 12$, with HSVrd-KATII). One week after spinalization normal saline, HSVrd or HSVrd-KATII was injected into the bladder walls of the three groups, respectively. Three weeks after virus injection, the urethral pressure profile (UPP) and continuous cystometry were performed under awake conditions and gene expression was evaluated in all of the SCI rats.

Results: In the HSVrd-KATII group, the maximum urethral closure pressure (Pclo.max), maximum voiding pressure (MVP), and the number and amplitude of non-voiding contraction (NVCs) were significantly decreased (34.7–39.1%, 46.7–56.2% and 31.5–32.5%, respectively), along with an increase in voiding efficiency (49.1–52.1%) compared with the sham and HSVrd groups. In addition, the levels of KATII protein and mRNA were significantly increased in the L6–S1 dorsal root ganglia (DRG) and L6–S1 spinal cord segments in the HSVrd-KATII group compared with the HSVrd group.

Conclusions: HSVrd vector encoding the *KATII* gene effectively improved DSD and detrusor overactivity by bladder-wall injection, perhaps by blocking N-methyl-D-aspartate receptors in the L6–S1 dorsal root ganglion and L6–S1 spinal cord.

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INTRODUCTION

The storage and periodic release of urine depends on the coordinated activity of two functional units in the lower urinary tract: (1) a reservoir (the urinary bladder); and (2) an outlet consisting of the bladder neck, the urethra and the striated muscles of the external urethral sphincter (EUS).¹ Spinal cord injury (SCI) at the thoracic or cervical levels disrupts voluntary control of voiding, as well as the normal reflex pathways that coordinate bladder and sphincter function.² Following SCI, the bladder is initially areflexic, but then becomes hyper-reflexic (detrusor overactivity [DO]) due to the emergence of a spinal micturition reflex pathway.² Voiding is inefficient, however, because the bladder-sphincter coordination is destroyed, appearing as simultaneous contractions of the bladder and urethral sphincter, and termed detrusor-sphincter dyssynergia (DSD).¹ These dysfunctions can lead to many problems, such as recurrent urinary tract infections, urinary stones, vesicoureteral reflux and even kidney impairment.³

The N-methyl-D-aspartate (NMDA) receptor is a type of ionotropic glutamatergic receptor that widely consist in the brain and spinal cord and have an important role in the normal micturition reflex pathways controlling the bladder and EUS.^{4,5} In SCI rats, MK-801, a non-competitive NMDA receptor antagonist, can decrease the activity of the bladder and EUS electromyography (EMG).^{4,6} Thus, the NMDA receptor can be regarded as target to treat neurogenic lower tract dysfunction. Kynurenic acid (KYNA) is the antagonist of the NMDA receptor, with a strong affinity towards the glycine B site.^{7,8} KYNA synthesis is catalyzed by kynurenine aminotransferase (KAT), the major isoform of which is KATII in the rat and human central nervous systems.⁹

Herpes simplex virus (HSV) vectors are non-toxic, neurotropic gene transfer tools that can express therapeutic genes after latent infections in specific neurons, can move in the retrograde and anterograde directions and disseminate trans-synaptically from neuron-to-neuron.¹⁰ Replication-defective herpes simplex virus

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(HSVrd) vector-mediated genes have been reported to treat interstitial cystitis, SCI-induced DO and DSD by injecting vectors into the bladder wall.^{3,11–13} In our previous study, we showed that HSVrd-encoding KATII suppressed DO, but increased voiding efficiency and decreased the maximum voiding pressure (MVP) in SCI rats.¹¹ Thus, we speculated that this therapeutic method not only inhibits DO, but also reduces urethral pressure and improves DSD. We did not, however, evaluate the change in urethral pressure.

In this study we hypothesized that injecting the HSV-mediated KATII gene into the bladder wall could not only inhibit DO, but also reduce urethral pressure and block NMDA receptors in the L6-S1 DRG, as well as the lumbosacral micturition center in SCI rats. Three weeks after viral injection we examined the continuous cystometry, urethral pressure profile (UPP) and HSVrd-KATII delivery by measuring the expression of KATII in L6-S1 spinal cord segments and the L6-S1 DRG.

MATERIALS AND METHODS

Viral vectors

The HSVrd-KATII vector was constructed by recombining the RG223277 plasmid (Origene Technologies, Beijing, China) human KATII cDNA into the ICP4-, ICP27- and ICP34.5-deleted HSVrd vector (SinoGenoMax, Beijing, China) with a 6-His tag derived from wild HSV-I virus (Figure 1). Both HSVrd and HSVrd-KATII express the viral reporter protein, RFP, under the control of an EF1a promoter. The vectors were propagated in the OG01 cell line (SinoGenoMax) derived from a Vero cell (CCL-81; ATCC, Manassas, VA, USA) that supplies ICP4 and ICP27 in trans.

Animal model

This study was approved by the Ethics Committee of the China Rehabilitation Research Center. Thirty-six adult female Sprague–Dawley rats (240–265 g) were used in this study protocol, which was approved by the Academy of Military Medical Science. The rats were randomly divided into three groups: sham group ($n = 12$ with normal saline); HSVrd group ($n = 12$, with HSVrd) and HSVrd-KATII group ($n = 12$, with HSVrd-KATII). All of rats were spinalized with complete transection at the T10 spinal cord level under anesthesia with 2% pentobarbital (30 mg kg^{-1}) by intraperitoneal injection. After the operation, the rats were treated with ampicillin sodium (100 mg kg^{-1} intramuscularly) for 5 days. The bladders of rats required abdominal compression three times daily until the micturition reflexes recovered (10–14 days), then once per day.

Viral injection

One week after spinal cord transection, the rats were anesthetized with pentobarbital (30 mg kg^{-1} intraperitoneally). The lower abdomen was opened via a midline laparotomy to expose the bladder. Then, the saline, HSVrd or HSVrd-KATII ($30 \mu\text{l}$, 2×10^7 plaque-forming units (PFU) in total) were separately injected into the bladder wall at five sites on the bladder wall around

the bladder base using a 30-gauge syringe ($10 \mu\text{l}$; Hamilton, Reno, NV, USA) under a microscope. After the operation, the rats were treated with ampicillin sodium (100 mg kg^{-1} intramuscularly) for 3 days.

Continuous cystometry and UPP

Three weeks after bladder-wall virus injection, the rats were anesthetized with 2% isoflurane and the abdomen was opened via a midline laparotomy to expose the bladder. The ureters were ligatured at the level of the aortic bifurcation. A PE-90 catheter was inserted into the bladder through the bladder dome for recording the intravesical pressure. The catheter was connected to a pressure transducer (MP150; Biopac, Goleta, CA, USA) and microinfusion pump (Stoelting, Wood Dale, IL, USA) through a three-way stopcock. After the rats recovered from anesthesia, the bladder was filled with normal saline at a rate of 0.08 ml min^{-1} at room temperature and 3 micturition cycles were recorded.

After cystometry, the bladder was emptied by compression. A PE-50 catheter with a lateral hole, which was connected to a pressure transducer and microinfusion pump through a 3-way stopcock, was inserted from the urethral meatus to the bladder for UPP recording. Before the urethral pressure recording, the bladder was infused with 0.05 ml of normal saline, then the microinfusion pump was regulated to 0.01 ml min^{-1} and the catheter was withdrawn at 2 mm s^{-1} . This course was repeated three times.

We regard the maximum urethral closure pressure (Pclo.max) as the index of urethral pressure. Pclo.max represents the difference between the maximum urethral and bladder pressures with the bladder at rest. The sum of infused saline was calculated as the bladder capacity. The voided saline was collected, then the residual volume and voiding efficiency (voiding efficiency = (voided volume/bladder capacity) $\times 100$) were calculated. The number and amplitude of non-voiding bladder contractions (NVCs), the MVP (cmH_2O), the time to first NVCs and the time to voiding were measured. NVCs were defined as an increase in rhythmic intravesical pressure $> 7 \text{ cm H}_2\text{O}$ from the baseline pressure without a release of fluid from the urethra.

Western blot quantification of KATII protein in the HSVrd and HSVrd-KATII groups ($n = 4$ each)

The L6-S1 spinal cord segment was homogenized in RIPA buffer (Sigma-Aldrich, Beijing, China) containing a protease inhibitor cocktail (Sigma-Aldrich). The homogenate was centrifuged at $12\,000 \text{ r.p.m}$ for 15 min at 4°C . For each sample, $30 \mu\text{g}$ of the protein extract was separated in a standard 12% Tris–HCl PAGE-Gel (Bio-Rad Laboratories, Shanghai, China), followed by electrophoresis at a concentrated gel electrophoresis voltage of 80 V and a gel electrophoresis separation voltage of 120 V . After electrophoresis, the proteins were transferred onto a nitrocellulose membrane (Bio-Rad Laboratories). The nitrocellulose blots were blocked for 1 h at room temperature with 5% dry milk in PBS with 0.05% Tween-20 (Sigma), then incubated with rabbit anti-human KATII antibodies (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA) overnight at 4°C , followed by horseradish peroxidase-conjugated goat anti-rabbit antiserum (1:3000; Santa Cruz Biotechnology) for 2 h at room temperature. Western blot analysis was performed using an ECL

Figure 1 Structures of HSVrd and HSVrd-KATII vectors. Both vectors express the tag protein of red fluorescent protein (RFP) under the control of EF1, a promoter, with the essential genes, ICP4 and ICP27, and both copies of the non-essential gene, ICP34.5, deleted. HSVrd-KATII expresses the therapeutic protein human KATII under the control of the human cytomegalovirus (CMV) immediate-early promoter with the 6-His tag.

detection system (Pierce, Beijing, China). Finally, the ratio of KATII-to-glyceraldehyde 3-phosphate dehydrogenase was calculated.

Qualification of KATII mRNA in the HSVrd and HSVrd-KATII groups ($n = 4$ each)

The L6-S1 DRG and L6-S1 spinal cord segments were rapidly removed, and total RNA was extracted from the L6-S1 DRG and spinal cord segments using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed into cDNA using a Script RT Reagent kit (KangJiaHongYuan Biotech, Beijing, China). Primers for KATII (forward primer: 5'-TAGTAACCAGAGGATG CAA-3'; and reverse primer: 5'-GCTGAAGAGAAGGATGCTC-3') and β -actin (forward primer: 5'-ACACCCGCCACCAGTTC-3'; and reverse primer: 5'-TGACCCATACCCACCATC-3') were used. KATII levels were determined with a quantitative real-time PCR system using a Script RT Reagent Kit (KangJiaHongYuan Biotech) and SYBR Premix Ex Taq II (Invitrogen). Amplification of cDNA was performed as follows: 95 °C for 5 min; then 40 cycles at 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30 s. Each tissue was examined three times, and melting-curve analysis was performed to confirm primer specificity. Standard curves were constructed from serial dilutions of cDNA in each tissue, and quantification of the samples was achieved using the standard curve. The ratio of KATII-to- β -actin mRNA was compared.

Statistical analysis

Data are expressed as the mean $\pm$ s.e. Statistical comparisons were performed using independent sample t-tests with SPSS 19.0 software (SPSS, Chicago, IL, USA). A P -value < 0.05 was considered statistically significant.

RESULTS

Comparisons of the urethral and bladder activities in the sham, HSVrd and HSVrd-KATII groups

Representative traces of UPP and continuous cystometry in the sham, HSVrd, and HSVrd-KATII groups are shown in (Figures 2,3 and 4; Table 1). In the sham and HSVrd groups, there were no significant differences in UPP and cystometric parameters (Table 1); however, in the HSVrd-KATII group, the MVP and the number and amplitude of NVCs were significantly decreased (34.7–39.1%, 46.7–56.2% and 31.5–32.5%, respectively), along with an increase in voiding efficiency (49.1–52.1%) compared with the normal saline and HSVrd-untreated groups. In addition, the maximum urethral closure pressure (Pclo.max) in the HSVrd-KATII group was significantly decreased by 41.1–44.7% ($P < 0.01$) compared with the sham and HSVrd groups. The voided volume was not significantly different among the three groups, but the voiding efficiency in the HSVrd-KATII group was

significantly increased by 49.1–52.1% ($P < 0.05$) compared with the HSVrd-untreated and HSVrd groups (Table 1).

Comparison of the level of KATII protein in the HSVrd and HSVrd-KATII groups ($n = 4$ each)

Using western blot analysis to examine the levels of KATII protein expression in the L6-S1 spinal cord segment and L6-S1 DRG. The ratio of KATII protein/glyceraldehyde 3-phosphate dehydrogenase in the L6-S1 spinal cord segment in the HSVrd-KATII group (0.41 ± 0.093) was significantly increased by 70.83% compared with the HSVrd group (0.27 ± 0.031 , $P < 0.05$, Figure 5b). In addition, the ratio of KATII protein in the L6-S1 DRG in the HSVrd-KATII group (0.39 ± 0.0150) was increased by 129% compared with the HSVrd group (0.17 ± 0.026 , $P < 0.01$, Figure 5a).

Comparison of the KATII level in the HSVrd and HSVrd-KATII groups ($n = 4$ each)

RT-PCR was used to determine the level of KATII mRNA expression in the L6-S1 spinal cord segment and L6-S1 DRG. The ratio of KATII -to- β -actin mRNA, which was used to assess KATII mRNA expression, was significantly increased by 88.3% in the HSVrd-KATII group (1.45 ± 0.11) compared with the HSVrd group (0.77 ± 0.07 ; $P < 0.01$, Figure 6a) in the L6-S1 DRG and 66% in the HSVrd-KATII group (0.95 ± 0.83) compared with the HSVrd group (0.57 ± 0.62 ; $P < 0.05$) in the L6-S1 spinal cord segment (Figure 6b).

DISCUSSION

The present study showed that KATII gene therapy by injecting HSVrd-KATII into the bladder wall inhibits DO and decreases voiding efficiency along with a decreased MVP, thus we speculate that the urethral pressure may also be decreased.¹¹ In the current study, the results indicated the following: (1) DSD was improved, as evidenced by a decreased maximum urethral closure pressure, the MVP, volume threshold-induced voiding and increased voiding efficiency. (2) DO was inhibited, as evidenced by the reduction in number and amplitude of NVCs and the prolongation of the time to first NVC.

The NMDA receptor is a type of ionotropic glutamatergic receptor, which is widely distributed in the brain and spinal cord, and has an important role in the normal micturition reflex pathways controlling the bladder and EUS.^{4,6} Previous studies have revealed that the intrathecal injection of MK-801 suppresses bladder contractions, including voiding pressure and increases the volume threshold for inducing voiding, as well as suppresses EUS EMG in normal and SCI

Figure 2 Recording the UPP. Pclo.max was calculated, as the index of urethral pressure. Pclo.max represents the difference between maximum urethral pressure and bladder pressure with the bladder at rest. (a) UPP in the HSVrd group; (b) UPP in the HSVrd-KATII group. Pclo.max was significantly decreased in the HSVrd-KATII group (b) compared with the HSVrd group (a).

Figure 3 Recording the continuous cystometry in SCI rats. (a) Continuous cystometry in the HSVrd group. (b) Continuous cystometry in the HSVrd-KATII group. In the HSVrd-KATII group, the MVP, the number and amplitude of NVCs, the time to first NVC, and the time to voiding in the HSVrd-KATII group (b) were all significantly decreased compared with the HSVrd group (a).

Figure 4 Comparison of Pclo.max (a), MVP (b) and the number (c) and amplitude (d) of NVCs in the sham, HSVrd and HSVrd-KATII groups. (a) The maximum urethral closure pressure (Pclo.max). (b) The MVP. (c) The number of NVCs. (d) The amplitude (d) of NVCs. In the HSVrd-KATII group, Pclo.max (a), MVP (b) and the number (c) and amplitude (d) of NVCs were all significantly decreased compared with the HSVrd and sham groups. * $P < 0.01$ and ** $P < 0.05$ when compared with the HSVrd and sham groups, respectively.

Table 1 Comparison of RUPP and cystometric parameters among sham, HSVrd and HSVrd-KATII groups.

	RUPP parameters				Cystometric parameters				
	Pclo.max (cmH ₂ O)	TF (min)	Number of NVCs	Amplitude of NVCs	MVP (H ₂ O)	VT (mO)	VW (ml)	T-FV (min)	VE (%)
Sham	6.30 ± 0.87	1.2 ± 0.58	12.78 ± 6.96	21.62 ± 5.28	40.01 ± 2.38	0.92 ± 0.23	0.069 ± 0.022	10.32 ± 2.26	6.42 ± 2.19
HSVrd	6.71 ± 0.85	1.44 ± 0.56	15.55 ± 5.90	21.93 ± 4.44	42.9 ± 2.95	0.95 ± 0.44	0.063 ± 0.017	12.21 ± 5.58	6.29 ± 3
HSVrd-KATII	3.71 ± 1.30**	2.6 ± 0.80**	6.81 ± 3.70**	14.81 ± 5.69*	26.11 ± 9.17**	0.61 ± 0.096**	0.062 ± 0.022	7.83 ± 1.44**	9.57 ± 2.97**

Abbreviations: HSVrd, replication-defective herpes simplex virus vector; KATII, kynurenine aminotransferase II; Pclo.max, the maximum urethral closure pressure; MVP, maximal voiding pressure; NVC, non-voiding bladder contraction; TF, time to the first NVC; T-FV, time to first voiding; VE, voiding efficiency; VT, voided volume. * $P < 0.01$ and ** $P < 0.05$ when the HSVrd-KATII group is compared with the HSVrd or sham group.

rats.⁵ This inhibitory effect on bladder activity is due to blocking NMDA glutamatergic receptors in the central nervous system. KAT-II is the astrocytic enzyme catalyzing the synthesis of KYNA, an endogenous inhibitor of the $\alpha 7$ -nicotinic receptor and the NMDA receptor (NMDAR), with a strong affinity towards the glycine site of the latter receptor.⁷ KYNA synthesis is catalyzed by KAT, the major isoform of which, KAT-II, is located in astrocytes in rats and humans.^{9,13}

The most important urinary tract afferent nerves are the myelinated (A δ) and unmyelinated (C) fibers, which convey information from receptors in the bladder and urethra to neurons in the spinal cord. In rats and cats with intact spinal cords, micturition is initiated by a supraspinal reflex pathway, which is triggered by A δ fibers.¹⁴ In normal cats, A δ fibers in the pelvic nerve and sacral dorsal roots of the cat respond to both passive distension as well as active contraction of the bladder;^{15,16} however, the majority of C-fibers are not sensitive to distension and contraction of the bladder, thus responding to chemical irritation and cold temperature. When C-fibers are stimulated, the afferent impulses in full and empty bladders are increased.² In chronic spinal cats, C-fibers rather than A δ fibers initiate voiding, and the spinal micturition reflex occurs with a short central delay (15 ms), in contrast to the long central delay (60 ms) of the reflex in cats with an intact spinal cord.¹⁷ It is thought that C-fibers, which usually do not respond to bladder distension, become mechanosensitive and initiate automatic micturition after SCI.¹⁸ In chronic SCI rats, due to a loss of control from the brain, micturition is initiated by a spinal reflex pathway, at which time the C-fibers contribute to bladder hyperactivity, leading to NVCs, as well as initiation of the voiding reflex by A δ fibers.^{1,2} Capsaicin, a C-fiber neurotoxin, blocks bladder contractions in chronic SCI cats, but does not block the bladder contraction reflex in intact cats.¹⁹ C-fibers are involved in initiating DO and capsaicin can suppress NVCs in SCI animals. In addition, abnormal sphincter activity is reduced by desensitization of C-fibers with capsaicin, indicating that C-bladder afferents contribute to DSD.²⁰ In the present study, the results (Figures 2,3 and 4; Table 1) of KATII gene therapy is similar to the effect of capsaicin, so we consider that this kind of KATII gene therapy could improve DSD and DO in SCI rats by decreasing C-fiber sensitivity.

In this study, KATII gene therapy mediated by injecting HSVrd-KATII into the bladder wall significantly suppressed the number and amplitude of NVCs, along with reducing the MVP in SCI rats (Figures 2,3 and 4; Table 1). Previous reports have shown that spinal glutamatergic transmission is important in both the afferent and efferent limbs of the spinal neural pathways controlling bladder activity.²¹ It has been proposed that glutamatergic transmission mediated by NMDA receptors is important in the spinal processing of afferent input from the bladder, and NMDA glutamatergic receptors have an important role in excitatory transmission in the descending pathway from the pontine micturition center to the spinal segmental circuitry involved in control of the urinary bladder.^{21,22} In addition, decreased NVCs and bladder contractility are assumed to reflect the suppression of afferent and efferent activity in the micturition reflex pathways, respectively.² Thus, KATII gene therapy in the current study possibly suppressed the afferent and efferent nerves of the micturition reflex. Combining the reported findings with the findings in the present study, we conclude that injecting HSVrd-KATII into the bladder wall of rats with SCI may suppress excitatory transmission in the afferent and efferent limbs of the reflex pathway from the lumbosacral spinal cord to the LUT.

Compared with the HSVrd group, the levels of KATII protein and mRNA were significantly increased in the HSVrd-KATII group in the

Figure 5 Comparisons of KATII protein levels in HSVrd and HSVrd-KATII groups. (a) KATII protein from L6-S1 DRG; (b) KATII protein from L6-S1 spinal cord. KATII protein in the L6-S1-spinal cord and L6-S1 DRG were significantly increased in the HSVrd-KATII group compared with the HSVrd group. * $P < 0.01$ and ** $P < 0.05$ when compared with the HSVrd and sham groups, respectively.

Figure 6 KATII mRNA expression in the HSVrd and HSVrd-KATII groups. (a) KATII mRNA from L6-S1 DRG; (b) KATII mRNA from the L6-S1 spinal cord segment. * $P < 0.01$ and ** $P < 0.05$ when compared with the HSVrd and sham groups, respectively.

L6-S1 DRG and L6-S1 spinal cord segment (Figures 5 and 6), indicating HSVrd-KATII expression of KYNA in the DRG and lumbosacral micturition center. It has been previously reported that after injection of labeled pseudorabies virus into the urinary bladder, urethra or EUS of the rat, pseudorabies virus is transported from peripheral efferent terminals to efferent neurons in the spinal cord and crosses multiple synapses to infect the interneuronal circuitry throughout the central nervous system, proving that spinal interneurons are involved in the lower urinary tract.^{23,24} Similarly, HSV vectors are non-toxic, neurotropic gene transfer tools that can express therapeutic genes after latent infection in specific neurons, can move in the retrograde and anterograde directions, and disseminate trans-synaptically from neuron-to-neuron.¹⁰ Our previous study proved that DO can be improved by injecting HSVrd-KATII in the bladder wall, and showed that increased exogenous KYNA after KATII gene delivery to DRG neurons released KYNA to the outside of the same or neighboring neurons to suppress afferent neuron activity by blocking NMDA receptors using whole-cell patch-clamp techniques.¹¹ We speculate that HSVrd-mediated KATII gene delivery in the parasympathetic preganglionic neurons and Onuf's nucleus in the

L6-S1 spinal cord segment blocks NMDA receptors in the lumbosacral micturition center as well as the DRG.

KYNA is a competitive antagonist of ionotropic glutamate receptors, preferentially inhibiting the obligatory glycine B site of the NMDA receptors.⁷ KYNA competitively combines in the glycine site of the NMDA receptors, blocking NMDA receptors and enhancing inhibition of glycinergic nerves on the micturition reflex because glycine is a major inhibitory neurotransmitter in the central nervous system, and inhibits the micturition reflex at the level of the lumbosacral cord in SCI rats.²⁵ According to previous reports, elevating the level of endogenous KYNA can reduce the level of extracellular glutamate in the rat brain.^{26,27} An increased level of KYNA after KATII gene therapy may partly reduce the level of extracellular glutamate in the spinal cord, partly suppressing overactivity of the bladder and urethral sphincter mediated by glutaminergic nerves in SCI rats.

Although the findings from this study highlighted possible clinical implications, we are aware of some limitations. First, we only performed UPP and continuous cystometry three weeks after virus injection, however, the long-term effects on SCI rats remain to be seen. Second, it has been reported that increased levels of KYNA in the

brain may contribute to neurodegenerative and psychiatric disorders, such as Alzheimer's and Parkinson's disease, schizophrenia and depression,²⁸ but in the current experiments, we only focused on the effects of this therapy in completely SCI rats, and possible side effects of this therapy were not evaluated in our present therapy. We still need to do more animal experiments to prove the safety and long-term effectiveness of KATII gene therapy in the future.

CONCLUSIONS

This study showed that KATII gene therapy by injecting HSVrd-KATII into the bladder wall can improve DSD and DO in SCI rats, likely by suppressing the C-fiber afferent and efferent limbs and blocking NMDA receptors, as well as blocking NMDA receptors and influencing neurotransmitter levels in the lumbosacral spinal cord and L6-S1 DRG.

DATA ARCHIVING

There were no data to deposit.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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