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SUPPRESSION OF DETRUSOR-SPHINCTER DYSSYNERGIA BY HERPES SIMPLEX VIRUS VECTOR-MEDIATED GLUTAMIC ACID DECARBOXYLASE GENE DELIVERY IN SPINAL CORD INJURES RATS

Hypothesis / aims of study

Micturition depends on the coordination between the bladder and external urethral sphincter. However, in the chronic phase of spinal cord injury (SCI), the bladder exhibits detrusor overactivity (DO) and bladder-sphincter coordination is impaired, leading to detrusor-sphincter dyssynergia (DSD).[1] Gamma-aminobutyric acid (GABA) is a major inhibitory transmitter in the central nervous system, and has an important role in the inhibitory regulation of micturition in SCI rats.[2] Hypofunction of inhibitory GABAergic neuronal activity in the spinal cord is also involved in the genesis of DO and DSD after SCI.[2] However, GABA-mediated therapeutic approaches have not been widely used because the therapeutic window of systemic application of GABA is modest and the applicable dose is limited by side effects. Thus, in the present study, we investigated the feasibility of gene therapy using replication-defective herpes simplex virus (HSV) vectors encoding glutamic acid decarboxylase (GAD), a GABA synthesis enzyme, for the treatment of DSD following SCI.

Study design, materials and methods

Adult female Sprague-Dawley rats with Th9-10 spinal cord transection were used. One week after spinalization, HSV vectors (total volume 40 µl, 5 x 10^8 plaque-forming units) expressing human GAD were injected to the bladder wall (HSV-GAD) via an abdominal midline incision under pentobarbital anesthesia. SCI rats without HSV injection (sham) and those injected with LacZ-encoding HSV vectors (HSV-LacZ) were used as controls.

(1) Simultaneous recordings of intravesical pressure and urethral perfusion pressure (UPP) were performed under an awake condition 3 weeks after viral injection, and cystometric parameters were compared among three groups.

(2) In order to examine the effect of GAD delivery, bicuculline ($GABA_A$ antagonist; 0.1 µg) or saclofen ($GABA_B$ antagonists; 1 µg) were administered through the intrathecal catheter at the level of L6-S1 spinal cord in the HSV-GAD group. Then cystometric parameters were compared before and after GABA receptor antagonists administration.

(3) Expression of GAD67 mRNA in L6-S1 dorsal root ganglia (DRG), where bladder afferent nerves originate, was assessed in HSV-GAD and HSV-LacZ groups. Then the ratio of GAD67 to β-actin mRNA was compared between two groups.

Results

(1) Comparisons between sham, HSV-LacZ, and HSV-GAD groups in intravesical pressure and UPP

There were not any differences in intravesical pressure among three groups. However, DSD characterized by urethral pressure increases during bladder contractions was significantly reduced ($p < 0.01$) in the HSV-GAD group compared with sham or HSV-LacZ groups (Table 1).

(2) Changes in intravesical pressure and UPP after GABA receptor antagonists in the HSV-GAD group

After intrathecal application of bicuculline, the amplitude of bladder contractions was significantly increased ($p < 0.05$), but intervals between bladder contractions and baseline bladder pressure did not change. The positive urethral pressure change during bladder contractions was also significantly increased ($p < 0.01$) after intrathecal bicuculline, and this value had not any differences when compared with those of sham and HSV-LacZ groups (Table 2).

After intrathecal application of saclofen, the positive urethral pressure change during bladder contractions was also significantly increased ($p < 0.05$), but urethral pressure rises during bladder contractions were still lower ($p < 0.05$) than those of sham and HSV-LacZ groups. The interval and amplitude of bladder contractions, baseline bladder pressure, and baseline urethral pressure did not change after intrathecal saclofen (Table 2).

(3) Comparisons of GAD67 mRNA level in L6-S1 DRG between HSV-LacZ and HSV-GAD groups

In the HSV-GAD group (3 weeks after viral injection), the GAD67 mRNA/β-actin mRNA ratio in L6-S1 DRG was significantly higher (0.52 ± 0.05, $p < 0.01$) compared with that of HSV-LacZ group (0.004 ± 0.001).

Table 1. Comparisons between sham, HSV-LacZ, and HSV-GAD groups in cystometric parameters

	Bladder contraction			Urethral contraction	
	Interval (min)	Amplitude (cm H_2O)	Baseline pressure (cm H_2O)	Urethral pressure change (cm H_2O)	Baseline pressure (cm H_2O)
Sham	0.47 ± 0.07	26.0 ± 2.8	17.0 ± 1.3	17.2 ± 2.5	21.4 ± 3.1
HSV-LacZ	0.34 ± 0.04	28.0± 3.9	14.7 ± 0.9	18.7± 1.9	13.2± 2.3
HSV-GAD	0.37 ± 0.03	21.6 ± 3.4	18.0 ± 1.4	4.0 ± 1.1**††	16.9 ± 4.1

Values are the mean ± SE. Significant differences when compared with sham are indicated by: ** $p < 0.01$.
Significant differences when compared with HSV-LacZ are indicated by: †† $p < 0.01$.

Table 2. Changes in bladder pressure and UPP after intrathecal administration of bicuculline or saclofen in the HSV-GAD group

	bicuculline		saclofen	
	before	after (0.1 μg)		
Bladder amplitude (cm H_2O)	23.0 ± 3.8	37.5 ± 4.6*	21.2 ± 3.6	25.4 ± 4.3
Bladder baseline pressure (cm H_2O)	18.2 ± 1.8	13.8 ± 2.3	17.6 ± 1.8	14.0 ± 2.1
Urethral pressure change (cm H_2O)	4.4 ± 1.2	19.2 ± 2.0**	4.6 ± 1.2	10.1 ± 2.5*
Urethral baseline pressure (cm H_2O)	14.3 ± 4.0	15.5 ± 2.6	13.8 ± 3.6	16.5 ±3.6

Values are the mean ± SE. Significant differences when compared with before application are indicated by: * $p < 0.05$; ** $p < 0.01$

Interpretation of results

In the HSV-GAD group, the urethral pressure increase during bladder contractions was 77-79% lower compared with sham and HSV-LacZ groups, suggesting that GAD gene delivery inhibits DSD. Intrathecal application of bicuculline completely reversed the decreased urethral pressure increase, while saclofen partially reversed it, suggesting that GAD gene delivery inhibits urethral activity predominantly through $GABA_A$ receptors. This inhibition was apparently mediated by increased production of GABA after HSV-GAD application because GAD67 mRNA level in the L6-S1 DRG was increased in the HSV-GAD group compared with the HSV-LacZ group.

Concluding message

This study provides the first evidence of the efficacy of GAD gene therapy using replication-defective HSV vector for DSD following SCI. HSV vectors can be efficiently transported to bladder afferent pathways and can inhibit DSD. Since hyperexcitability of C-fiber bladder afferents are reportedly involved in DSD during voiding after SCI,[3] it is assumed that GAD gene therapy could inhibit activation of C-fiber bladder afferent pathways to exert its effects predominantly through $GABA_A$ receptors. Therefore, the novel GAD gene therapy using replication-defective HSV vectors would be useful for the treatment of DSD by restoring impaired GABA mechanisms in patients with SCI.

References

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Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
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