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# Improvement of detrusor overactivity by ivermectin-mediated activation of double mutant glycine receptors delivered by herpes simplex virus (HSV) vectors in mice with spinal cord injury (SCI)

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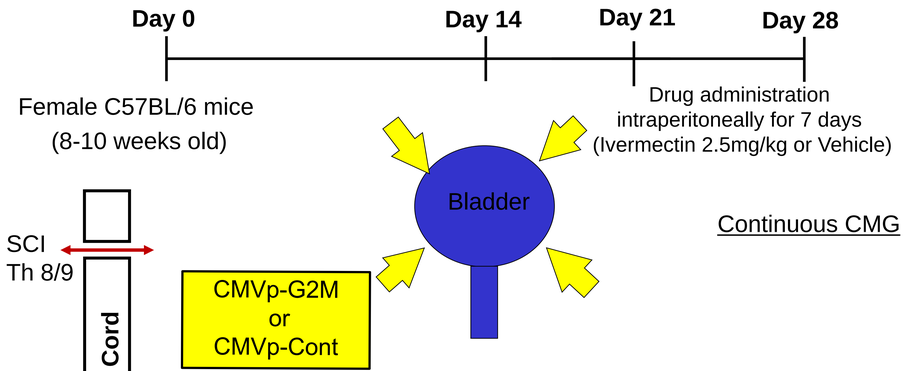
## Introduction and Objective:

Chronic spinal cord injury (SCI) rostral to the lumbosacral level induces both storage and voiding dysfunctions due to detrusor overactivity (DO) during the storage phase and detrusor-sphincter dyssynergia (DSD) during the voiding phase, respectively [1]. Glycine is one of major inhibitory neurotransmitters in the central nervous system (CNS), and activation of glycine receptors is known to induce a chloride ion influx to cause hyperpolarization in neuronal cells. It has also been reported that the spinal glycinergic mechanism is downregulated in the lumbosacral spinal cord; thereby inducing DO after SCI in rats [2]. However, it is likely that activation of endogenous glycine receptors may lead to systemic adverse events because of their ubiquitous distribution in the CNS. Therefore, this study aimed to develop a “druggable” approach targeting bladder afferent pathways by using gene delivery of the double mutant glycine (G2M) receptor, in which the receptor sensitivity to, ivermectin (IVM) an anthelmintic used in filariasis, is increased with elimination of sensitivity to glycine [3]. For this purpose, we utilized replication-deficient herpes simplex virus (HSV) vectors because they have a natural property allowing transfection to sensory pathways following peripheral inoculation, which could offer an organ-specific treatment, and investigated whether IVM-induced activation of G2M receptors delivered by HSV vectors driven by non-selective cytomegalovirus (CMV) promoter to bladder afferent pathways improves storage and voiding dysfunctions in mice with SCI.

## Materials and Methods:

Adult female C57BL/6 mice were used, and the spinal cord was completely transected at the Th8/9 level. We prepared replication-deficient HSV vectors encoding G2M receptors driven by non-specific cytomegalovirus (CMV) promoter (CMVp-G2M) and control HSV vectors encoding mCherry marker protein (CMVp-Cont). Two weeks after SCI, CMVp-G2M or CMVp-Cont vectors were inoculated into the bladder wall. One week after vector inoculation, IVM (2.5mg/kg/day) or vehicle (Veh) was administered intraperitoneally for 7 days. Then, at 4 weeks after SCI, continuous cystometrograms (CMG) were recorded under an awake condition to evaluate non-voiding contractions (NVCs).




CMVp-G2M; G2M receptors driven by CMV promoter = 1.10 x $10^{10}$ PFU/mL
CMVp-Cont; mCherry driven by CMV promoter = 1.11 x $10^{10}$ PFU/mL

SCI mice were divided into 4 groups:
(1) CMVp-G2M inoculation with IVM group (CMVp-G2M-IVM; n = 6)
(2) CMVp-G2M inoculation with Veh group (CMVp-G2M-Veh; n = 6)
(3) CMVp-Cont inoculation with IVM group (CMVp-Cont-IVM; n = 4)
(4) CMVp-Cont inoculation with Veh group (CMVp-Cont-Veh; n = 4)

## Discussion:

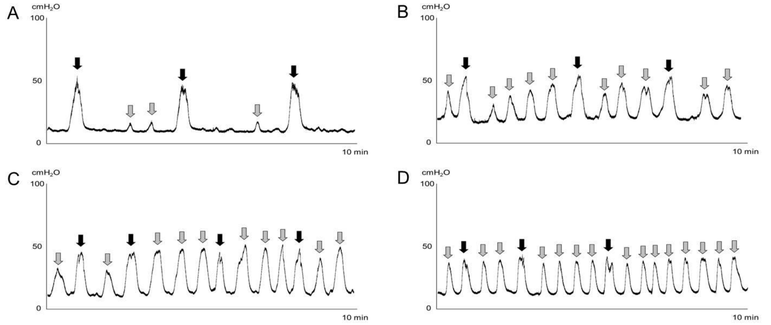
It has been documented that SCI initially induces areflexic bladder and urinary retention, and then later develops DO and DSD during voiding due to the reorganization of reflex pathways in the spinal cord as well as alterations in the properties of bladder afferent neurons [1]. The results in this study indicate that IVM-induced activation of G2M receptors expressed in bladder afferent neurons by bladder wall inoculation of HSV-G2M vectors driven by CMV promoter improved DO evident as a reduction of NVCs and inefficient voiding caused by DSD. Thus, it seems likely that HSV vector-mediated gene delivery of G2M designer receptors to bladder afferent pathways could offer a new approach using an exogenously applied synthetic ligand, IVM, to improve SCI-induced storage and voiding dysfunctions.

## Conclusion:

Gene therapy with replication-deficient HSV vectors encoding IVM-sensitive, mutant glycine receptors with exogenous ligand application could be a novel chemogenetic “druggable” treatment that can avoid systemic adverse events for neurogenic bladder dysfunction in SCI.

## Results:

The number of NVCs (0.30 ± 0.12/min) in the CMVp-G2M-IVM group was significantly smaller, compared to other three groups (0.86 ± 0.12, 1.25 ± 0.14 and 1.27 ± 0.26 in Groups 2-4, respectively) (Figure 1).




| | NVCs/min | P Value |
|---|---|---|
| CMVp−G2M−IVM | 0.30 ± 0.12 | |
| CMVp−G2M−Veh | 0.86 ± 0.12 | < 0.01 |
| CMVp−Cont−IVM | 1.25 ± 0.14 | < 0.01 |
| CMVp−Cont−Veh | 1.27 ± 0.26 | < 0.01 |

Figure 1: The representative CMG traces; CMVp-G2M-IVM group (A), CMVp-G2M-Veh group (B), CMVp-Cont-IVM group (C) and CMVp-Cont-Veh group (D). Black arrows; voiding contraction, grey arrows; non-voiding contraction. Table shows the number of non-voiding contractions (NVCs) per min in each group.

**References:**

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**Conflict of interest:**
None

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