Neuroprotective Effects of Valproic Acid in a Rat Model of Cauda Equina Injury

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BACKGROUND: **Histone deacetylase inhibitors, including valproic acid (VPA), are promising therapeutic interventions in neurological disorders and play an important role in synaptic activity and neuronal function.**

METHODS: **A total of 30 rats were randomly allocated to 3 groups: sham, control, and VPA. The rats in the VPA and control groups received laminectomy at the L4 level of the vertebrae and silicone gel implantation into the epidural spaces L5 and L6. Rats in the sham group only received laminectomy at the L4 level of vertebrae without any implantation. VPA (300 mg/kg in saline) was administered 2 hours before the surgery. After the surgery, the VPA group received further VPA injections at 300 mg/kg twice a day for 1 week. The same volume of saline was injected in the control group. Neurobehavioral tests using the Basso, Beattie, Bresnahan scale and the oblique board test were performed for 1 week starting at 2 hours before surgery up to day 7 after surgery. At day 7 after surgery, tissues from the compressed cauda equina (L5-L6) were subjected to hematoxylin and eosin, luxol fast blue, or immunofluorescence staining, whereas the terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick-end label assay staining was performed on the tissue from the dorsal root ganglions and the lumbar segment of the spinal cord proximal to the compressed cauda equina (L5-L6).**

RESULTS: **The behavioral results suggested a significant improvement in the lower limb motor function in the VPA group compared with controls ($P < 0.05$). Furthermore, histologic assessment revealed a significant reduction in nerve fibers showing Wallerian degeneration and demyelinating lesions in the VPA group, in addition to an increased myelination compared with the control group ($P < 0.05$). The terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick-end label assay staining revealed a significant decrease in the number of apoptotic neurons in the spinal cord anterior horn and dorsal root ganglions in the VPA group compared with controls ($P < 0.05$).**

CONCLUSIONS: **Our data demonstrated that VPA could alleviate cauda equina injury, reduce apoptotic cells, and improve motor recovery, suggesting a neuroprotective effect in acute cauda equina syndrome.**

INTRODUCTION

The cauda equina syndrome (CES) is a neurological disorder characterized by catastrophic consequences for the patients, including low back pain, sciatica, lower limb weakness, lower limb and saddle region sensory disturbances, and sphincter dysfunction.[1] As a common clinical disorder, CES results from the dysfunction of sacral and lumbar nerve roots in the spinal canal caused by herniated lumbar disk, spinal stenosis, tumor, trauma, spinal epidural hematoma, iatrogenic causes, or infections.[2] Cauda equina is the bridge of the spinal cord and peripheral nerves, connecting spinal cord neurons and

Key words
- Cauda equina syndrome
- Histone acetylation
- Histone deacetylase inhibitor
- Neuroprotective effect
- Valproic acid

Abbreviations and Acronyms
BBB: Basso, Beattie, Bresnahan
CEC: Cauda equina compression
CES: Cauda equina syndrome
DRG: Dorsal root ganglion
HDAC: Histone deacetylase
HDACi: Histone deacetylase inhibitor
H&E: Hematoxylin and eosin
LFB: Luxol fast blue staining
MBP: Myelin basic protein
TUNEL: Terminal deoxynucleotidyl transferase-mediated biotinylated UTP nick-end label
VPA: Valproic acid

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dorsal root ganglion (DRG) pseudounipolar neurons.[3] It is different from peripheral nerves that are surrounded by multiple layers of continuous connective tissue, including epineurium, nerve bundle membrane, and endoneurium. The cauda equina is surrounded only by a layer of endoneurium.[3] Due to this lack of protective structures, the cauda equina is vulnerable to mechanical compression injury. It is widely known that patients with CES have a poor prognosis.[4,5] Surgical treatment can only relieve the cauda equina compression (CEC), reduce secondary injury, and provide limited space for functional recovery. However, once the patients with CES show saddle region sensory disturbances and intestinal and sexual dysfunction, the prognosis of surgical treatment becomes very poor. Most patients present with residual bladder or anal sphincter dysfunction.[6,7] Therefore, developing an effective treatment to deal with CES and improve the prognosis is urgent. Efforts to develop alternative therapeutic agents, such as methylprednisolone, simvastatin, and hydrogen sulfide, have focused on the reduction of degeneration and recovery of neurological functions. Although these treatments have been, at least partially, effective, the question remains concerning their benefits versus their risks.[8-10]

As one of the most widely studied epigenetic modifications during the process of nerve pathology, histone modification has the potential to influence many fundamental biological processes.[11] Histone acetylation is one of the posttranslational histone modifications that serve as epigenetic tags. Histone deacetylases (HDACs) play a key role in the homeostasis of histone acetylation and in regulating transcriptional machinery. An array of neurological disorders has been linked to histone hypoacetylation and associated transcriptional dysfunction. By restoring the balance of histone acetylation, adjusting transcriptional dysfunction, and up-regulating neurotrophic genes, HDAC inhibitors (HDACis) have been viewed as a promising intervention for neurological disorders.

Valproic acid (VPA) is a well-established drug used to manage various neurological and psychiatric disorders, and is identified as an inhibitor of HDAC. It reduces the neuronal death induced by lipopolysaccharide, excitotoxicity, or aging by enhancing histone acetylation and adjusting gene transcription.[12-15] VPA exerts neuroprotective effects in an array of neurological disorders, including stroke, traumatic brain injury, Huntington disease, Parkinson disease, Alzheimer disease, amyotrophic lateral sclerosis, spinal muscular atrophy, and spinal cord injury.[14,16-21] In addition, it has been found that VPA can stimulate neurite growth and promote axonal regeneration in some neurological conditions.[22-24]

HDACis have emerged as the basis of a novel therapy for neurological disorders. However, there are few investigations observing the therapeutic potential of HDACis such as VPA in CES. The present study exploited a rat model of CEC to investigate the effects of VPA on functional recovery, apoptosis, and neurite outgrowth.

METHODS

Experiment Design

A randomized controlled animal experiment design was used. Rats were randomly assigned to 3 groups (sham, control, and VPA), each comprising 10 rats. Cauda equina injury was induced in rats using silicon compression, and an injection of VPA (300 mg/kg in saline) was administered 2 hours before the surgery. After surgery, the VPA group received additional injections of VPA (300 mg/kg) twice a day for 1 week.[14,22] The same volume of saline was injected in the control group. Rats were assessed in several neurobehavioral tests during 1 week extending from 2 hours before the surgery to day 7 after the surgery. Subsequently, histopathologic examinations were performed on day 7 after surgery.

Animals

Male Sprague-Dawley rats (weight, 250–300 g; age, 9–10 weeks) were purchased from the Experimental Animal Center of Shanghai Slack Company with License Number of SCXK (Shanghai) 2012-0002. The animal procedures used in this study were approved by the Animal Ethics Committee of Shanghai Second Military Medical University. Rats were housed in a 12:12-hour light:dark cycle with free access to food and water. They were acclimatized for 1 week before the start of the experiment and were deprived of food 12 hours before surgery.

Surgical Procedure

The CEC rat model was established as previously described.[10,25,26] Briefly, rats were anesthetized using an intraperitoneal injection of 10% chloral hydrate (0.3 mL/100 g). After confirming the efficacy of anesthesia by toe pinching, rats were depilated on their dorsal spine and placed in prone position. An incision was made over the spinal midline at the L4 to S2 level to reveal the L4 to L5 lamina. Subsequently, laminotomy was performed with L4 lamina. In addition, the ligamentum flavum was removed between L5 and L6. A piece of a silicone block (length, 10 mm; width, 1 mm; thickness, 1 mm) was inserted into the epidural space under the L5 to L6 vertebrae. After surgery, the wound was irrigated with phosphate-buffered saline. Sham-operated rats underwent the same surgery procedure without the silicon insertion. The rat's bladders were pressed 3 times daily with artificial urination; the abdominal cavities were massaged to promote defecation and prevent urinary retention and obstruction.

Behavioral Assessment

After baseline testing at 2 hours before the surgery, the rats were tested once a day for 1 week after surgery. Neurobehavioral performance was evaluated using the Basso, Beattie, Bresnahan (BBB) Locomotor Rating Scale[27] and oblique board test.[28] Two observers, who were unaware of the experimental procedures, performed the tests and recorded the scores.

Histologic Assessments

Three groups each comprising 5 rats were anesthetized and used for histologic assessments on day 7 after surgery. The compressed parts (L5-L6) of the cauda equina, DRGs, and lumbar segments of spinal cord proximal to the compressed cauda equina were dissected and postfixed overnight in 4% paraformaldehyde. Subsequently, these tissues were embedded in paraffin and sections were cut at 4 μm. For histologic analyses, sections of the compressed parts of the cauda equina were subsequently stained with hematoxylin and eosin (H&E),[10] Luxol fast blue staining (LFB), and immunofluorescence staining. Sections of the DRGs and lumbar segments of spinal cord proximal to the compressed