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Clinical Article

The Effect of Phosphodiesterase-4-Specific Inhibitor in the Rat Model of Spinal Nerve Ligation

Sung Hoon Kim, M.D., Ph.D.,[1] Bit-Na-Ri Park, M.D.,[1] Seok Won Kim, M.D., Ph.D.[2]

Department of Rehabilitation,[1] College of Medicine, Wonju Christian Hospital, Yonsei University, Wonju, Korea
Department of Neurosurgery,[2] College of Medicine, Chosun University, Gwangju, Korea

Objective : Peripheral neuropathy is characterized by hyperalgesia, spontaneous burning pain, and allodynia. The purpose of this study was to investigate the effect of rolipram, a phosphodiesterase-4-specific inhibitor, in a segmental spinal nerve ligation model in rats.
Methods : Both the L5 and L6 spinal nerves of the left side of the rats were ligated. Phosphodiesterase-4 inhibitor (rolipram) and saline (vehicle) were administered intraperitoneally. We measured mechanical allodynia using von Frey filaments and a nerve conduction study.
Results : The mechanical allodynia, which began to manifest on the first day, peaked within 2 days. Multiple intraperitoneal injections of rolipram ameliorated the mechanical allodynia. Furthermore, an intraperitoneal administration of rolipram improved the development of pain behavior and nerve conduction velocity.
Conclusion : This study suggests that the phosphodiesterase-4 inhibitor, rolipram, alleviates mechanical allodynia induced by segmental spinal nerve ligation in rats. This finding may have clinical implications.

Key Words : Peripheral nerve injury · Allodynia · Rolipram.

INTRODUCTION

Spinal radiculopathy and peripheral neuropathy may generate a syndrome characterized by spontaneous pain, and exaggerated responses to light touch and temperature stimuli. The polyneuropathy is an incapacitating complication of chronic drug consumption, characterized by allodynia and pain, primarily in the lower extremities, and is poorly relieved by available treatments[23]. Depending on the criteria and patient selection, the incidence of peripheral neuropathy has been reported to range from 10% to 50%[22]. The pathogenesis of drug-induced neuropathy is an axonal neuropathy characterized by wallerian degeneration of the axons and a reduction in the myelination of nerve fibers[36]. Controversy surrounds the pathogenic role of trauma or drugs in the development of neuropathy. Studies on rat models have suggested that many causative factors have a direct neurotoxic effect on neuronal organelles and the spinal cord system[5,16,24]. Oxidative stress and inflammatory stress are known to play a very pivotal role in the experimental animal models of neuropathic pain. Lee et al.[18] suggested that reactive oxygen species are critical to the development and maintenance of capsaicin-induced pain, particularly in the process of central sensitization in the rat nervous system. Padi and Kulkarni[26] demonstrated that chronic administration of minocycline when started early, before peripheral nerve injury, could ameliorate the development of neuropathic pain by inhibiting the release of proinflammatory cytokines and oxidative and nitrosative stress in mononeuropathic rats. A significant increase in lipid peroxidation and decrease in the activity of antioxidant enzymes (superoxide dismutase and catalase) have been observed in the sciatic nerves of diabetic rats with established neuropathic pain[30]. Dina et al.[6] demonstrated that hyperalgesia is present in an established model of peripheral neuropathy in the rat and that inflammatory process and protein kinase signaling play a pivotal role in the enhanced nociception. A important action of cAMP is activation of transcription factors including c-AMP-responsive element binding (CREB) protein and nuclear factor-kB (NF-kB) p50[12]. Phosphorylation of CREB stimulates transcription of cell survival genes[20]. Phosphorylation of NF-kB p50 subunit suppresses transcription of genes associated with inflammation, especially the pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-Iβ)[4,12,19,33]. Thus, in the current study, we tested the hypothesis that rolipram, a selective inhibitor of cAMP-specific phosphodiesterase

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• Address for reprints : Seok Won Kim, M.D., Ph.D.
Department of Neurosurgery, School of Medicine, Chosun University,
588 Seoseok-dong, Dong-gu, Gwangju 501-717, Korea
Tel : +82-62-220-3126, Fax : +82-62-227-4575
E-mail : ns64902@hanmail.net

(PDE), would play a pivotal role in improving mechanical allodynia and nerve conduction velocity in segmental spinal nerve ligation-induced neuropathic pain in rats.

MATERIALS AND METHODS

Experimental animals

Sixteen male adult Sprague-Dawley rats weighing 200-350 g were used in this study. The animals were housed in two groups in plastic cages with soft bedding and free access to food and water. All animals were acclimated in their cages for 1 week before any experiments were performed. All experimental protocols were approved by the Institutional Animal Care and Use Committee at our institure and carried out in accordance with the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Segmental spinal nerve ligation model and drug treatment protocol

Under sodium pentobarbital anesthesia (40 mg/kg, i.p.), the rat was placed in a prone position and the left paraspinal muscles were separated from the spinous processes at the L4-S2 level. The L6 transverse process was carefully removed to identify the spinal nerves. The left L5 and L6 spinal nerves were ligated with 6-0 silk thread. The PDE-4-specific inhibitor, rolipram (Sigma, St. Louis, MO, USA), was first dissolved and then gently mixed with 0.9% physiological saline to a final 10% v/v solution. Physiological saline was used as the vehicle for the control group. According to their specific study group, animals received rolipram or vehicle once daily for a period of three weeks.

Behavioral tests for mechanical allodynia

Behavioral tests were conducted blindly so that the experimenter who conducted the tests did not know the nature of the experimental application. The behavioral tests measured were foot withdrawal thresholds (as an indicator of mechanical allodynia) in response to mechanical stimuli applied to the left hind paws. For each test, the animals were placed in a plastic chamber (9×9×30 cm) and habituated for at least 10 minutes. The chamber was placed on top of a mesh screen, so that mechanical stimuli could be administered to the plantar surface of the left hind paws. Thresholds were determined by the up-down method[7] using a set of von Frey monofilaments (von Frey filament values : 3.65, 3.87, 4.10, 4.31, 4.52, 4.74, 4.92, and 5.16; equivalent to : 0.45, 0.74, 1.26, 2.04, 3.31, 5.50, 8.32, and 14.45 g values). Gram (g) means bending force of a set of von Frey monofilaments. A von Frey filament was applied perpendicularly to the most sensitive areas of the plantar surface at the center area of paw or the base of the third or fourth toes with sufficient force to bend the filament slightly for 3-4 seconds. An abrupt withdrawal of the foot during stimulation or immediately after stimulus removal was considered to be a positive response. The first stimulus was always the 4.31 filament. When there was a positive response, the next lower filament was used, and when no response was observed, the next higher filament was applied. This testing pattern continued until responses to the sixth von Frey stimuli from the first change of response (either higher or lower than the first stimulus depending on whether the first response was negative or positive) were measured. The responses were then converted to a 50% threshold value using the formula : 50% threshold _ 10(X _ kd)/ 104, where X is the value of the final von Frey hair used in log units, k is the tabular value for the pattern of positive or negative responses, and d is the mean differences between stimuli in log units (0.22). When positive or negative responses were still observed at the 3.65 or 5.16 filament, values of 0.3 or 18.6 g were assigned, respectively, by assuming a value of _0.5 for k in these cases.

Electrophysiological findings

A Nicolet Viking IV (Nicolet Biomedical Ins., USA) was used for recording the sensory evoked potential (SEP). The SEP is an objective and reliable electrophysiological measurement that reflects the status of the sensory pathways. All SEP responses were obtained from stimulation of an anesthetized, unrestrained rat. Rats were placed in the prone position on a plastic board with the active electrode implanted 2.5 mm posterior and 2.8

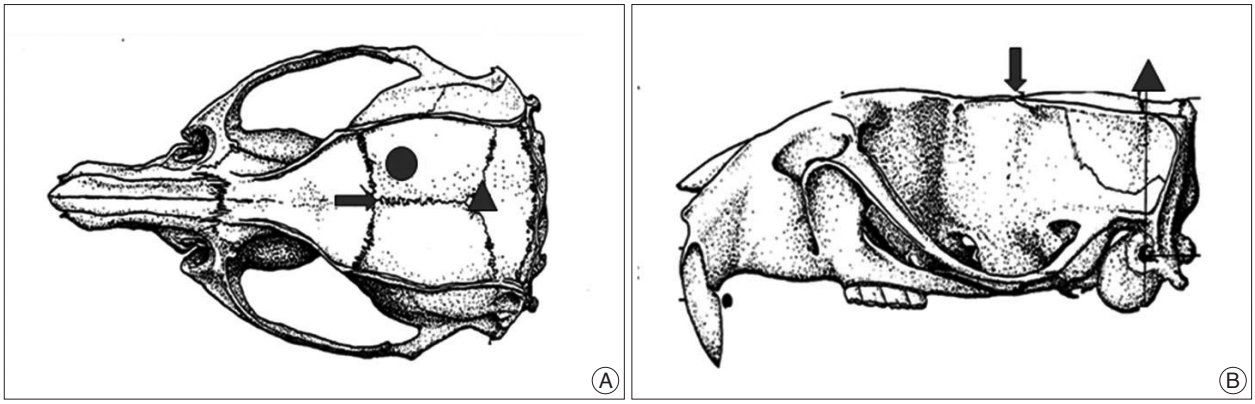

Fig. 1. A dorsal (A) and lateral (B) schematic of the rat skull with electrode placement for the SEP study. The arrow, triangle and circle indicate the bregma, lambda and recording electrode, respectively. SEP : sensory evoked potential.

mm laterally to the bregma and the reference electrode was implanted in the mid frontal bone (Fig. 1). The SEP responses were elicited by activation of the tibial nerve in the left leg. The ground electrode was placed subcutaneously between the stimulation and recording electrode. The left limb was stimulated by positive current pulses of 1.3-1.9 mA magnitude, 0.2 msec duration, and 3 Hz frequency. The signal-to-noise ratio was improved by ensemble averaging of 500 stimulus locked sweeps. The first peak latency was recorded. All animals were evaluated at day 1, 2, 3, 4, 8, 13, 17, and 21.

Statistical analysis

Statistical analyses were done using two-way repeated-measures analysis of variances with two-repeated factors followed by Tukey post hoc test for the experiment of Latin square design or two-way repeated-measures analysis of variances with one repeated time factor followed by Tukey post hoc tests. Results are presented as means_SEMs and analyzed using the Sigma Stat program (Systat Software, Inc., Chicago, IL, USA). In all cases, $p<0.05$ was considered statistically significant.

RESULTS

Segmental spinal nerve ligation model

Mechanical allodynia threshold

The baseline mechanical threshold for all rats before operation was 17.1 g, which was the maximal cutoff point. We used the left hind paw as the site to measure the nociceptive threshold. Beginning at day 1, there was a significantly greater decrease in nociceptive threshold, a peak at 2 days and then a plateau until week 3, the final week of the study ($p<0.05$) (Fig. 2).

SEP responses

Beginning on day 1, there was an increase in latency in the vehicle control group, and the increase was statistically significant after 2 days ($p<0.05$) (Fig. 3).

Role of rolipram, specific PDE-4 inhibitor

We found that an intraperitoneal injection of the phosphodiesterase-4 inhibitor, rolipram, improved mechanical behavior and latency of SEP, compared with the vehicle-injected group ($p<0.05$) (Fig. 2, 3).

DISCUSSION

The neurologic effects of numerous physical traumas and drugs are complex, encompassing both the central and peripheral nervous system[28,34,37]. In the peripheral nervous system, the neurologic effect is generally small-fiber death and painful neuropathy[2,6]. Over time, the pain far outweighs the analgesia, producing a neuropathic pain syndrome with symptoms that have been described as "like tearing flesh off the bones"[3]. In the recent years, peripheral neuropathy due to anticancer drugs and ethanol consumption in western industrialized countries has increased among women with an associated increase in the rates of alcohol-related health problems[8,11]. Thus, understanding the basis of clinical expression of neuropathy is an issue of growing importance and adequate treatment of this symptom may require therapeutic strategies. PDE4 inhibitors have been reported to reduce both proinflammatory cytokine levels, including TNF-α and IL-1β[1,27], molecules involved in free radical production and oxidative stress, such as iNOS and COX-2, as well as immune cell infiltration into the nervous system[29]. Free radicals are derivatives of molecular oxygen and nitrogen, and in-

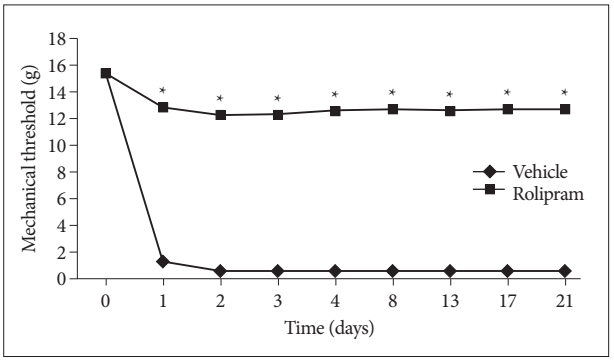

Fig. 2. The time courses of mechanical threshold in L5, 6 spinal nerve ligation induced-neuropathy. Rolipram was injected intraperitoneally once a day. The vehicle group received an equal volume of normal saline. Note that spinal nerve ligation with vehicle application significantly decreased the mechanical threshold at the beginning of day 1. Rolipram significantly ameliorated the mechanical threshold decrease in comparison to the vehicle-injected group ($p<0.05$). Asterisks indicate values significantly different ($p<0.05$) from corresponding vehicle values using a two-way repeated measures analysis of variance with a repeated time factor, followed by the Tukey post hoc test.

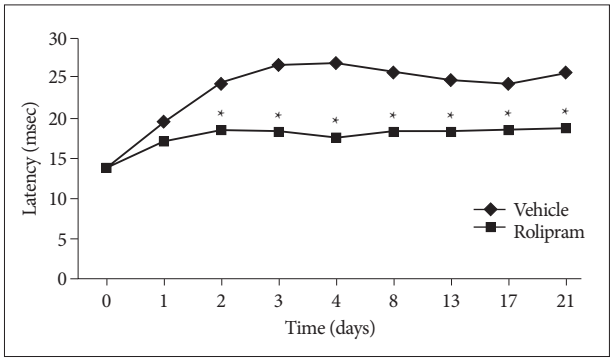

Fig. 3. The time courses of SEP in L5, 6 spinal nerve ligation induced-neuropathy. Rolipram was injected intraperitoneally once a day. The vehicle group received an equal volume of normal saline. Note that spinal nerve ligation with vehicle application significantly increased the latency at the beginning of day 1, peaked on day 2 and then reached a plateau until the final day of the study. Rolipram significantly ameliorated the latency increase compared with the vehicle-injected group ($p<0.05$). Asterisks indicate values determined significantly different ($p<0.05$) from the corresponding vehicle values using a two-way repeated measures analysis of variance with a repeated time factor, followed by the Tukey post hoc test. SEP : sensory evoked potential.

clude superoxide, hydroxyl radical, hydrogen peroxide, and peroxynitrite[17]. These molecules are ubiquitously present in the body and participate in many normal cellular processes including ion transport, transcription, neurotransmission, and neuromodulation[17]. Sources of free radicals include mitochondrial oxidative metabolism to produce adenosine triphosphate and several enzymes such as xanthine oxidase, phospholipase A2, cytochrome P450, monoamine oxidase, and tyrosine hydroxylase. Free radicals are normally removed by antioxidant systems including superoxide dismutase, catalase, glutathione, glutathione peroxidase, ascorbate, and α-tocopherol. Thus, their levels are precisely controlled by antioxidant systems. However, in pathologic conditions, levels of free radicals may increase due to the increased production or decreased antioxidants level[9,10]. Two proinflammatory cytokines released after a central nervous lesion are TNF-α and IL-1β. Numerous studies have documented rapid increases in TNF-α and IL-1β levels after traumatic brain and spinal cord injury[15,35]. IL-1β synergistically acts with TNF-α to induce nerve cell death. These proinflammatory cytokines stimulate inflammatory cells to release damaging reactive oxygen and nitrogen species, raise glutamate levels to excitotoxic levels, impair the ability of glia cells to buffer extracellular potassium, compromise the nervous system and attract inflammatory cells into the nervous system[13,14,21,31,32]. Once initiated, the inflammatory cascade becomes a toxic positive-feedback loop, further exacerbating nervous system pathology. In the models of nervous system injury, several studies have demonstrated that restoration of cAMP levels improve outcome. In the animal model, application of rolipram to inhibit the degradation of cAMP promotes axon sparing and results in functional outcomes[25]. In this study, we found that rolipram can prevent degradation of cAMP and improve mechanical behavior and latencies of SEP.

CONCLUSION

Our study shows that rolipram ameliorates pain behavior as measured by mechanical allodynia and SEP in an animal model of segmental spinal nerve ligation. Further, rolipram did not induce gastrointestinal problems or sedation at the given doses, so the behavioral changes are interpreted as analgesia. These results raise several questions for future research to identify the longterm effect and optimal dose of the PDE-4-specific inhibitor rolipram.

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