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Carnosic acid attenuates neuropathic pain in rat through the activation of spinal sirtuin1 and down-regulation of p66shc expression

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ABSTRACT

Background: It has been reported that carnosic acid (CA) exhibits a range of biological activities including hepatoprotective, antioxidant and anti-inflammatory. However, the effect of carnosic acid in neuropathic pain remained elusive.
Methods: A neuropathic pain model of chronic constriction injury (CCI) was established in adult male Sprague–Dawley rats. Mechanical withdrawal threshold (MWT) and thermal withdrawal latency (TWL) were recorded, and western blot was performed to detect sirtuin1 and p66shc content.
Results: Intrathecal administration of carnosic acid attenuated mechanical allodynia and thermal hyperalgesia in rats following chronic constriction injury. Interestingly, carnosic acid analgesic effect was positively associated with spinal sirtuin1 activation; however, p66shc was inhibited by carnosic acid in the spinal cord. In additional, sirtuin1 inhibitor EX-527 reversed the anti-nociceptive effect of carnosic acid.
Conclusions: Carnosic acid is effective in the treatment of the established CCI-induced pain. It may be possible that spinal sirtuin1 activition by carnosic acid attenuates neuropathic pain through a mechanism involving the down-regulation of p66shc expression.

1. Introduction

Neuropathic pain is a debilitating pain state, which is often caused by injury to the nervous system arising from bone compression in cancer, diabetes mellitus, infection, autoimmune disease, or physical injury (Baron, 2006). It is characterized by the presence of spontaneous ongoing and evoked pain, with the latter presenting as allodynia (pain elicited by a nonnoxious stimulus) or hyperalgesia (increased pain response to anoxious stimulus) (Finnerup and Jensen, 2006; Finnerup et al., 2007), which are refractory to conventional analgesics. From general population studies, neuropathic pain affects up to 5% of the population (Bouhassira et al., 2008; Torrance et al., 2006). These led us to discovery new approaches to treat chronic pain-related disorders.

Previous studies suggest that neuropathic pain might result from multiple pain-related cellular and molecular alternations in primary afferent or spinal cord after nerve injury (Uchida et al., 2010a,b; Adilakshmi et al., 2012). One of the characteristic alterations in gene modification is abnormal histone acetylation, which has been shown to contribute to the development of neuropathic pain. It has been demonstrated that the impact of abnormal histone acetylation, can be modulated by Sirtuin1 (Sirt1) (Uchida et al., 2013; Kiguchi et al., 2012). Sirt1, a member of the highly conserved nicotinamide adenine dinucleotide–dependent class III histone deacetylases, plays important roles in liver protection, genomic stability, inflammation via deacetylation of its target proteins (Zhu et al., 2013; Hao and Haase, 2010; Michan and Sinclair, 2007). Additionally, in a SOD1 G93A transgenic mouse model of amyotrophic lateral sclerosis, the expression of Sirt1 in lumbar segment of the spinal cord was changed (Lee et al., 2012), indicating that Sirt1 may regulate the pathogenesis of neuropathic pain.

Interestingly, recent works suggest that the regulation by Sirt1

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may involve p66shc. Sirt1 transgenic diabetic mice exhibit decreased expression of p66shc (Chen et al., 2012) and Sirt1 -mediated p66shc inhibition is crucial for improving hepatocyte function during rats with liver ischemia/reperfusion injury (Yan et al., 2014). P66shc is an isoform of the mammalian adapter protein ShcA and an increasing number of reports demonstrated that it contributes to physiological and pathophysiological process. Notably, p66shc knockout mice did not have age-dependent increases in pain sensitivity as compared with the corresponding wild-type mice (Berry et al., 2007). However, the role of p66shc in neuropathic pain remains unknown.

Thus we reasoned that Sirt1 inhibiting p66shc after peripheral nerve injury may lead to effective approach to the treatment of neuropathic pain. Carnosic acid (CA) is a major constituent of the labiate herbal plant rosemary and it has been found to exhibit a range of biological activities including hepatoprotective, antioxidant, anti-inflammatory (Yan et al., 2014; Ibarra et al., 2011). However, little research has been conducted regarding the therapeutic efficacy of CA in neuropathic pain. Here, we explored whether CA, which shared a structural similarity with Sirt1 activators like resveratrol and quercetin (Chung et al., 2010), attenuated neuropathic pain at the spinal level and we aimed to elucidate whether its function was linked to Sirt1 and p66shc. To address these issues, the effect of intrathecal injection of CA on the development of neuropathic pain in rats following chronic constriction injury (CCI) was examined. Changes in the expression of spinal Sirt1 and p66shc relation to neuropathic pain were examined by using Western blot. The effects of CA on the regulation of Sirt1 and p66shc were evaluated. Finally, we characterized the nociceptive behavior of CCI rats using Sirt1 inhibitor EX-527(6-chloro-2, 3, 4, 9-tetrahydro-1-H-carbazole-1-carboxamide), which is more potent and selective than other current Sirt1 inhibitors (Napper et al., 2005), before intrathecal injection of CA to further elucidate the role of Sirt1 in CA analgesic effect.

2. Materials and methods

2.1. Experimental animals

All experimental protocols were performed according to the standards established in the Guide for the Care and Use of Laboratory Animals published by the Institute of Laboratory Animal Resources of the National Research Council (United States) and were approved by the Institutional Animal Care and Use Committee of Wenzhou Medical University (Wenzhou, China). 162 male Sprague Dawley rats weighing 230–250 g were purchased from SLAC Experimental Animals Company of Shanghai (Shanghai, China) and housed in a temperature-controlled (22 °C) colony room under a 12 h/12 h light/dark cycle regime, with food and water available ad libitum at the Experimental Animal Center of the Wenzhou Medical University. All behavioral testing was performed during the light cycle between 10:00 AM and 2:00 PM.

2.2. Lumbosacral intrathecal surgery

Lumbosacral intrathecal (IT) catheters was inserted into the lumbar enlargement according to the method described previously (Milligan et al., 1999). The lumbar approach was utilized because it avoided pressure on the spinal cord. Briefly, rats were anesthetized with 4% chloral hydrate (400 mg/kg, intraperitoneally [IP]). A rectangular area of the skin above the L2-L6 lumbar vertebral was shaved and sterilized with alcohol. A 2 cm longitudinal skin incision was made. At a point 15 mm caudal to the L5 – L6 lumbar gap, a 20 gage guide cannula was inserted at an angle about 20°into the muscle and was carefully advanced rostrally along the dorsal surface of the L6 vertebra, as the angle of the syringe was reoriented to about 15° until the cannula was entered the subarachnoid space. The correct intrathecal localization was characterised by a sudden slight flick of the tail or a paw retraction. A PE-10 polyethylene catheter, with an outer diameter of 0.5 mm was used. Submerged in water of 60 °C, one end of the catheter was reduced in diameter by stretching it to about 150% of the original length. A 14-cm length of PE-10 catheter with volume of approximately 7 μl was easily inserted through the cannula and advanced about 3 cm beyond the tip of the inserted cannula to reach the level of the lumbar enlargement. The cannula was then removed, leaving the catheter in place. A loop knot was tied in the catheter and slightly tightened. The knot itself was anchored to white collagenous tissue with a sterile suture of 4–0. The catheter was tunneled under the skin, appearing close to the base of the tail. A bead 10 cm from the tip was made on the catheter to prevent the catheter from being dislocated. After injecting 10 u1 phosphate-buffered saline (PBS), the catheter was sealed by melting the end. Our preliminary study used 10 μl Evans blue dye to ensure the presence of Evans blue dye over cauda equina at the lumbosacral level. The skin incision was then closed with silk sutures. The cannulated rats were allowed to recovery from anesthesia and were housed individually. Those rats exhibiting postoperative neurological deficits or poor grooming were excluded from the experiments. All catheter placements were verified upon completion of behavioral testing by visual inspection. Data were analyzed only from animals in which the catheter tip was verified as being at the lumbosacral spinal level.

2.3. Chronic constriction injury model

One or two days after days after the catheter implantation, rats received either chronic constriction injury of the sciatic nerve or sham surgery. CCI model as one of neuropathic pain models was prepared based on previous description (Bennett and Xie, 1988). Rats were anesthetized with 4% chloral hydrate (400 mg/kg, intraperitoneally [IP]) during surgical procedures. The right sciatic nerve was exposed at mid-thigh level. Around the dissected nerve, four ligature knots (4–0 chromic gut) were performed loosely. Intervals among ligature knots were about 1 mm. The tension of the constriction was guided by the occurrence of a short flick of the ipsilateral hind limb. Skin wound was closed with 4–0 sterile silk. For sham operation, the sciatic nerve was exposed but not manipulated. The rectal temperature of all the rats was maintained by constant temperature blanket during surgical procedures until the rats recovered from anesthesia.

2.4. Drugs injections

Injection of drugs was conducted in restrained animals placed in a pile of soft towels during drug administration. The animals were kept horizontal during injections. After an initial more than 30 s acclimation period, the sealed tip was cut and the drugs were microinjected intrathecally with a 10-μl microsyringe in a volume of 10 μl followed by a DMSO flush. The drug was injected about a 1 min period into the subarachnoid space. The needle of microsyringe was left in place for further 15 s before withdrawl. The time taken for the total procedure was about 3 min. Carnosic acid and EX-527 were purchased from Sigma (St. Louis, MO) and were dissolved in 10 μl 10% DMSO (100% DMSO diluted in PBS). The doses of carnosic acid and EX-527 were selected according to previous reports (Peck et al., 2010; Satoh et al., 2008; Shao et al., 2014) and our preliminary experiments.