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Original Reports

Alendronate Attenuates Spinal Microglial Activation and Neuropathic Pain

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Abstract: **Many derivatives of bisphosphonates, which are inhibitors of bone resorption, have been developed as promising agents for painful pathologies in patients with bone resorption-related diseases. The mechanism for pain relief by bisphosphonates remains uncertain. Studies have reported that bisphosphonates could reduce central neurochemical changes involved in the generation and maintenance of bone cancer pain. In this study, we hypothesized that bisphosphonates would inhibit spinal microglial activation and prevent the development of hyperalgesia caused by peripheral tissue injury. We investigated the effects of alendronate (a nitrogen-containing bisphosphonate) on the development of neuropathic pain and its role in modulating microglial activation in vivo and in vitro. Intrathecal and intraperitoneal administration of alendronate relieved neuropathic pain behaviors induced by chronic constriction sciatic nerve injury. Alendronate also significantly attenuated spinal microglial activation and p38 mitogen-activated protein kinase (MAPK) phosphorylation without affecting astrocytes. In vitro, alendronate downregulated phosphorylated p38 and phosphorylated extracellular signal regulated kinase expression in lipopolysaccharide-stimulated primary microglia within 1 hour, and pretreatment with alendronate for 12 and 24 hours decreased the expression of inflammatory cytokines (tumor necrosis factor α, and interleukins 1β and 6). These findings indicate that alendronate could effectively relieve chronic constriction sciatic nerve injury–induced neuropathic pain by at least partially inhibiting the activation of spinal microglia and the p38 MAPK signaling pathway.**

Perspective: ***Alendronate could relieve neuropathic pain behaviors in animals by inhibiting the activation of spinal cord microglia and the p38 MAPK cell signaling pathway. Therapeutic applications of alendronate may be extended beyond bone metabolism–related disease.***

Key words: *Alendronate, chronic constriction injury, microglial activation, p38 mitogen-activated protein kinase, inflammatory cytokines.*

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Neuropathic pain is a debilitating disease often caused by peripheral nerve injury. It can result in an increased response to noxious stimuli (hyperalgesia) and stimuli that do not normally provoke pain (allodynia).[71] It is known that central sensitization is involved in development of chronic pain. Several lines of evidence have verified that glia play an important role in central hyperactive states as well as in the multiple alterations in dorsal horn neurons after nerve injury.[63-65]

Microglia, the immune and defense glia, respond very quickly to noxious stimulus and enter an activated state. Histological analysis shows proliferation and hypertrophy of these activated microglia in neuropathic pain.[11,13,15] These cells not only exert morphological changes but also exhibit some functional alterations when activated compared with when in a quiescent state. Activated microglia can release multiple proinflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, which play an important role in hypersensitivity in neurons.[6,12,23] It is well known that development of tactile allodynia after nerve injury depends on p38 mitogen-activated protein kinase (MAPK), 1 of 4 subgroups of the MAPK family.[28,30,44,65] The activation of p38 MAPK is observed in activated microglia, but not in neurons nor in astrocytes in the dorsal horn after nerve injury.[65] Furthermore, phosphorylated p38 (p-p38) is known to regulate the synthesis of numerous proinflammatory cytokines via transcriptional regulation. This cascade is also known to play an essential role in the initiation of neuropathic pain as well as in long-term maintenance of central sensitization in the spinal cord. Thus, suppression of microglial activation and p-p38 activation in microglia might prevent the development of hyperalgesia caused by peripheral nerve injury.[20,35,47]

Alendronate (ALN) is one of the nitrogen-containing bisphosphonates and is commonly used to treat osteoporosis.[5,31,70] Several reports have shown that ALN treatment of bone metabolism disorders contributes to pain alleviation.[45,57] Therefore, it is important to elucidate how ALN potentially modulates pain control mechanisms and chronic pain development. Bisphosphonates are pyrophosphate analogues in which the oxygen bridge has been replaced by a carbon with various side chains phosphorus-carbon-phosphorus.[24] It prevents bone absorption by inducing apoptosis in osteoclasts or by inhibiting osteoclast activation.[42,46] Meanwhile, ALN can inhibit the bacteria-induced protein tyrosine phosphatase (PTP) activities, such as CD45, at very low 50% inhibiting concentration values.[54] CD45 is a novel molecular therapeutic target to inhibit MAPK activation in microglia.[74] We previously reported that CD45 expression was significantly induced in activated microglia in the lumbar spinal cord after intraplantar injection of formalin or in models of nerve injury.[15,36] Because osteoclasts and microglial cells are members of the monocyte phagocytic system and derived from hematopoietic stem cells,[1] ALN may also exert a similar effect on microglia. In our study, we hypothesized that ALN would attenuate activation of spinal microglia to modulate pain transmission by inhibiting CD45 and/or downstream cell signaling molecules such as MAPKs. In this study, we used an in vivo chronic constriction injury (CCI) model to investigate the effects of ALN on mechanical and thermal hypersensitivity and activation of glial cells. We also used an in vitro microglia culture to test whether ALN could directly inhibit microglial activation.

Methods

Animals and Treatment

Adult male Sprague Dawley rats weighing 280 to 300 g were used. The animal room was artificially lighted from 7:00 AM until 7:00 PM. The experimental protocol was approved by the Institutional Animal Care and Use Committee of Peking University. The surgical procedure was performed aseptically using pentobarbital (50 mg/kg, intraperitoneal) anesthesia. CCI rats underwent loose ligation of the common sciatic nerve according to the method of Bennett and Xie.[3] Sham rats received the same surgical procedure, except the nerve ligation was omitted.

Drug Delivery and Nociceptive Behavioral Testing

A PE10 intrathecal catheter was implanted in rats at the level of the lumbar enlargement approximately a week before CCI surgery according to the method described previously.[72] Rats exhibiting postoperative neurological deficits (eg, paralysis) or poor grooming (2 of 44) were excluded from the experiments. ALN was purchased from Sigma-Aldrich (St. Louis, MO) and was dissolved in sterilized .9% saline. ALN of different doses or sterilized .9% saline was injected intrathecally in a 10-μL volume followed by a 10-μL saline flush once a day from day 1 to day 7. Rats (n = 42) were randomly placed into 6 groups: sham with vehicle sterilized .9% saline (n = 7), sham with ALN (20 μg/kg, n = 7), CCI with vehicle (n = 7), CCI with ALN (20 μg/kg, n = 7), CCI with ALN (10 μg/kg, n = 7), and CCI with ALN (4 μg/kg, n = 7).

For the peritoneal injection, animals were randomly placed into 4 groups. ALN was dissolved in saline at concentrations of .05 mg/mL, .5 mg/mL, and 2.5 mg/mL and administered peritoneally as follows: CCI with vehicle (n = 8), CCI with ALN (.1 mg/kg, n = 8), CCI with ALN (1 mg/kg, n = 8), and CCI with ALN (5 mg/kg, n = 8). Treatment was initiated at the first day after CCI surgery and was terminated on day 7. The day of the CCI surgery was marked as day 0. Peritoneal administration of ALN occurred from day 1 to day 7.

Animals were habituated to the behavior test setting 2 days before beginning the experiment and allowed at least 20 minutes to acclimatize before the testing. The testing procedure for thermal hyperalgesia was performed according to a previously published method.[21] Temperature was set to have the baseline latency at approximately 10 to 12 seconds. The maximum time allowed was 20 seconds to prevent tissue damage. The mechanical allodynia test procedure was developed according to the report from Tal and Bennett.[60] A range of von Frey filaments were applied to the plantar surface of each hind paw, starting from the middle weighted filament (10 g). Each filament was tested 5 times, and the final gram weight of the filament was recorded if animals responded positively to the filament 1 to 2 times in the trial. The cutoff force was 20 g. The behavior testing operator was blinded to treatment procedures. All