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Original Reports

Dorsal Root Ganglion Infiltration by Macrophages Contributes to Paclitaxel Chemotherapy-Induced Peripheral Neuropathy

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Abstract: **Chemotherapy-induced peripheral neuropathy (CIPN) is a disruptive and persistent side effect of cancer treatment with paclitaxel. Recent reports showed that paclitaxel treatment results in the activation of Toll-like receptor 4 (TLR4) signaling and increased expression of monocyte chemoattractant protein 1 (MCP-1) in dorsal root ganglion cells. In this study, we sought to determine whether an important consequence of this signaling and also a key step in the CIPN phenotype was the recruitment and infiltration of macrophages into dorsal root ganglia (DRG). Here, we show that macrophage infiltration does occur in a time course that matches the onset of the behavioral CIPN phenotype in Sprague-Dawley rats. Moreover, depletion of macrophages by systemic administration of liposome-encapsulated clodronate (clophosome) partially reversed behavioral signs of paclitaxel-induced CIPN as well as reduced tumor necrosius factor α expression in DRG. Intrathecal injection of MCP-1 neutralizing antibodies reduced paclitaxel-induced macrophage recruitment into the DRG and also blocked the behavioral signs of CIPN. Intrathecal treatment with the TLR4 antagonist lipopolysaccharide-RS (LPS-RS) blocked mechanical hypersensitivity, reduced MCP-1 expression, and blocked the infiltration of macrophages into the DRG in paclitaxel-treated rats. The inhibition of macrophage infiltration into DRG after paclitaxel treatment with clodronate or LPS-RS prevented the loss of intraepidermal nerve fibers (IENFs) observed after paclitaxel treatment alone. These results are the first to indicate a mechanistic link such that activation of TLR4 by paclitaxel leads to increased expression of MCP-1 by DRG neurons resulting in macrophage infiltration to the DRG that express inflammatory cytokines and the combination of these events results in IENF loss and the development of behavioral signs of CIPN.**

***Perspective:** This paper shows that activation of innate immunity by paclitaxel results in a sequence of signaling events that results in the infiltration of the dorsal root ganglia by activated macrophages. Macrophages appear to drive the development of behavioral hypersensitivity and the loss of distal epidermal nerve fibers, and hence play an important role in the mechanism of paclitaxel-related neuropathy.*

Key words: *Cancer, neuropathic pain, TLR4, MCP-1, TNF-α.*

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Chemotherapy-induced peripheral neuropathy (CIPN) represents a dose-limiting adverse effect of cancer treatment that affects as many as 50% of patients with cancer treated with single agents and more than 75% when combination therapies are used.[30,35] CIPN is observed after the administration of several types of drugs commonly used for the treatment of many of the most common solid and hematologic malignancies, including vinca alkaloids, taxanes, platinum derivatives,

and bortezomib.[17,30,61] Furthermore, CIPN represents a clinical problem that is steadily on the increase as the number of long-term survivors of cancer increases. CIPN most often presents as a sensory neuropathy with complaints of burning and shooting pains, tingling, and numbness and is observed as a length-dependent neuropathy with a stocking and glove distribution; common analgesics aimed at reducing the painful symptoms are often ineffective.[8,9,16,20,25,26,39] The anticancer modes of action for the various chemotherapeutic drugs are largely understood, but the neurotoxic mechanisms contributing to the selectivity of the damage to sensory neurons alone and the clinical severity of CIPN remain unclear.[17,30,61] There are no pharmacologic or other means available to inhibit the occurrence of CIPN. Hence, dose reduction and withdrawal of the offending agent is the only option to slow the development of CIPN, potentially affecting optimal treatment.[17,30,61]

Paclitaxel is one of the most effective chemotherapeutic drugs, widely used for the treatment of solid tumors such as ovarian, breast, and non–small-cell lung carcinoma; it is associated with the development of CIPN.[35] Although the specific mechanisms underlying the development of paclitaxel CIPN remain undefined, there are several lines of evidence indicating that engagement of innate immunity plays a key role.[36,40-42,47] For example, application of minocycline, an inhibitor of proinflammatory cytokine release, prevents mechanical allodynia induced by paclitaxel,[15,43] and we have shown that intrathecal treatment with the Toll-like receptor 4 (TLR4) antagonist lipopolysaccharide-RS (LPS-RS) transiently reversed preestablished CIPN mechanical hypersensitivity and prevented the development of any behavioral signs of CIPN when given as a protective agent during chemotherapy.[42] Further, it was shown that paclitaxel treatment induces increased expression of monocyte chemoattractant protein 1 (MCP-1) in DRG and spinal cord and blockade of MCP-1/CCR2 signaling by anti-MCP-1 antibody or CCR2 antisense oligodeoxynucleotides significantly attenuated paclitaxel-induced mechanical hypersensitivity, as well as the loss of distal intraepidermal nerve fibers (IENFs).[63] MCP-1/CCL2 is a potent chemokine that regulates migration and infiltration of monocytes/macrophages,[22] and macrophages have been observed in dorsal root ganglia (DRG) and the spinal dorsal horn in models of paclitaxel-induced CIPN.[43,47] Because a characteristic role of innate immunity involves monocyte/macrophage secretion of proinflammatory mediators, including tumor necrosis factor α (TNF-α), interleukin 1β (IL-1β), IL-6, MIP-1α, MIP-1β, and MCP-1, which are widely recognized to contribute to an array of persistent pain states,[27,34,45] we hypothesized that paclitaxel treatment activates innate immunity, resulting in macrophage recruitment to DRG, and that these then drive the induction and maintenance of paclitaxel-induced peripheral hypersensitivity.

Materials and Methods

Animals

Adult male Sprague-Dawley rats (weighing 250–300 g; Harlan, Houston, TX) housed in a 12-hour light/dark cycle with free access to food and water were used in all experiments. The studies were approved by the institutional animal care and use committee at the University of Texas M. D. Anderson Cancer Center and were performed in accordance with the National Institutes of Health guidelines for use and care of laboratory animals.

Paclitaxel CIPN Model

Animals were treated with paclitaxel as previously described.[7,14,23,42,48] Briefly, 6 mg/mL stock pharmaceutical grade paclitaxel (TEVA Pharmaceuticals, Inc, North Wales, PA) was diluted with sterile .9% saline to 1 mg/mL and given at a dosage of 2 mg/kg intraperitoneally every other day for a total of 4 injections (days 1, 3, 5, and 7). Control animals received an equivalent volume of the vehicle only, which consisted of equal amounts of Cremophor EL (Sigma-Aldrich, St. Louis, Missouri) and ethanol diluted with saline to reach a concentration of vehicle similar to the paclitaxel concentration. Rats were observed carefully for any abnormal behavioral changes every other day after the treatment. Signs of peripheral neuropathy with a similar phenotype to that in patients have been validated in this non–tumor-bearing animal model of paclitaxel CIPN by multiple investigators.[7,14,23,42,48]

Intrathecal Treatment

Intrathecal drug delivery was performed by lumbar puncture as previously described.[21,42,62] Rats were anesthetized with isoflurane (2.5%) and injected between the L4-L5 intervertebral space using a 12.7-cm (.5-inch) 30-gauge needle connected to a luer-tipped Hamilton syringe. Correct subarachnoid positioning of the tip of the needle was verified by tail-flick. TLR4 antagonist LPS derived from *Rhodobacter sphaeroides* (LPS-RS, 20 μg in 20 μL phosphate-buffered saline [PBS]; InvivoGen, San Diego, CA) or anti-MCP-1 neutralized antibody (200 μg/mL, 20 μL per application; AbD Serote, Raleigh, NC) or equal amount of nonspecific IgG (rabbit IgG; Jackson ImmunoResearch, West Grove, PA) were delivered intrathecally 24 hours before the first injection of paclitaxel and continued once daily for the next 7 days for a total of 8 injections (days 0–7). Intrathecal injection was given 30 minutes before paclitaxel when both drugs were administered on the same day.

Intravenous Injection of Clodronate

The macrophage toxin clodronate in liposomes (clophosome-A, 7 mg/mL clodronate disodium) or control liposome (FormuMax, Sunnyvale, CA) were intravenously administrated to 24 rats (12 versus 12) with the volume of .8 mL on day 7 and day 10 in paclitaxel-treated rats.

Mechanical Withdrawal Threshold

Mechanical withdrawal threshold was tested before, during, and after paclitaxel treatment by an