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Toll-Like Receptor 4 Signaling Contributes to Paclitaxel-Induced Peripheral Neuropathy

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Abstract: **This paper tests the contribution of the toll-like receptors, TLR4 in particular, in the initiation and maintenance of paclitaxel-related chemotherapy-induced peripheral neuropathy. TLR4 and its immediate downstream signaling molecules—myeloid differentiation primary response gene 88 (MyD88) and toll/interleukin 1 receptor domain–containing adapter-inducing interferon-β (TRIF)—were found to be increased in the dorsal root ganglion (DRG) using Western blot by day 7 of paclitaxel treatment. The behavioral phenotype, the increase of both TLR4 and MyD88, was blocked by cotreatment with the TLR4 antagonist lipopolysaccharide–*Rhodobacter sphaeroides* during chemotherapy. A similar, but less robust, behavioral effect was observed using intrathecal treatment of MyD88 homodimerization inhibitory peptide. DRG levels of TLR4 and MyD88 reduced over the next 2 weeks, whereas these levels remained increased in spinal cord through day 21 following chemotherapy. Immunohistochemical analysis revealed TLR4 expression in both calcitonin gene-related peptide–positive and isolectin B4–positive small DRG neurons. MyD88 was only found in calcitonin gene-related peptide–positive neurons, and TRIF was found in both calcitonin gene-related peptide–positive and isolectin B4–positive small DRG neurons as well as in medium- and large-size DRG neurons. In the spinal cord, TLR4 was only found colocalized to astrocytes but not with either microglia or neurons. Intrathecal treatment with the TLR4 antagonist lipopolysaccharide–*R. sphaeroides* transiently reversed preestablished chemotherapy-induced peripheral neuropathy mechanical hypersensitivity. These results strongly implicate TLR4 signaling in the DRG and the spinal cord in the induction and maintenance of paclitaxel-related chemotherapy-induced peripheral neuropathy.**

***Perspective:** The toll-like receptor TLR4 and MyD88 signaling pathway could be a new potential therapeutic target in paclitaxel-induced painful neuropathy.*

Key words: *Neuropathy, DRG, spinal cord, TLR4, MyD88, TRIF, LPS-RS.*

Paclitaxel is the front-line chemotherapeutic agent used to treat many of the most common solid tumors, including those of the breast, ovary, and lung.[34] Peripheral neuropathy is the major dose-limiting side effect of paclitaxel and can force dose reduction or even discontinuation of therapy, thus affecting survival in cancer patients.[20] In addition, chemotherapy-induced peripheral neuropathy (CIPN) often persists long after cancer treatment is completed and is commonly refractory to current treatment strategies, thus affecting rehabilitation, the return to productivity, and quality of life in cancer survivors.[9,10] Neuropathic pain in general is considered to involve an important role of glial cells and proinflammatory immune responses in the underlying basic pathophysiology,[62] and evidence implicates similar mechanisms in CIPN.[7,8] Of special interest in this regard is the observation that paclitaxel engages the same signaling pathway via toll-like receptor 4 (TLR4) as the very well-known proinflammatory agent lipopolysaccharide (LPS).[12] Paclitaxel binds to and activates TLR4 in macrophages resulting in activation of the nuclear

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factor-kappa B (NF-κB) signal path and the induction of proinflammatory cytokine expression identical to that produced by LPS.[12]

TLR4 is expressed on the surface of innate immune cells, small primary afferent neurons,[63] and central nervous system cells, including microglia and astrocytes.[11] Thus, there is a plausible link between TLR4 and the production of proinflammatory cytokines in neural tissue, which could contribute to behavioral hypersensitivity following exposure to chemotherapy drugs. A previous study indicated that TLR4 in the central nervous system plays a role in the development of behavioral hypersensitivity in a rodent model of neuropathic pain.[60] Rats that lacked functional TLR4 and those that received intrathecally administrated TLR4 antisense oligonucleotides showed attenuated nerve injury–induced behavioral hypersensitivity in both the initiation and maintenance phases.[6] There are no data at present regarding the potential role of TLR4 signaling in paclitaxel-induced neuropathic pain. This report explores the effects of paclitaxel chemotherapy on the expression of TLR4 in neural tissue and the effects of antagonists to TLR4 and its immediate downstream signals, myeloid differentiation primary response gene 88 (MyD88) and toll/interleukin 1 receptor domain–containing adapter-inducing interferon-β (TRIF), in reducing paclitaxel-induced behavioral hypersensitivity.

Methods

Animals

Male Sprague-Dawley rats weighing 250 to 300 g (Harlan, Houston, TX) were used to establish the neuropathic pain model. Rats were housed in temperature- and light-controlled (12-hour light/dark cycle) conditions with food and water available ad libitum. All 169 rats used in the study were included in the behavioral analysis portions and then used in follow-up pharmacologic, immunohistochemistry, or Western blot analysis. The numbers of rats in each of these studies are detailed in the relevant sections. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Texas MD Anderson Cancer Center and were performed in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Every procedure was designed to minimize discomfort to the animals and to use the fewest animals needed for statistical analysis.

Paclitaxel-Induced Neuropathy Model

Rats were treated with paclitaxel (TEVA Pharmaceuticals, Inc, North Wales, PA) as previously described[69] based on the protocol of Polomano et al.[48] In brief, pharmaceutical-grade Taxol was diluted with sterile saline from the original stock concentration of 6 mg/mL (in 1:1 Cremophor EL:ethanol) 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), resulting in a final cumulative dose of 8 mg/kg. Control animals received an equivalent volume of the vehicle only, which consisted of equal amounts of Cremophor EL and ethanol diluted with saline to reach a concentration of vehicle similar to the paclitaxel concentration. No abnormal spontaneous behavioral changes were noted during or after paclitaxel or vehicle treatment.

TLR4 and MyD88 Antagonist Administration

To assess the role of the TLR4–MyD88 signaling pathway in maintaining paclitaxel-induced neuropathic pain, 20 μg of the TLR4 antagonist LPS derived from *Rhodobacter sphaeroides* (LPS-RS) in 20 μL phosphate-buffered saline (PBS; InvivoGen, San Diego, CA) or 500 μM of MyD88 homodimerization inhibitory peptide (MIP) in PBS (Imgenex, San Diego, CA) was injected intrathecally by L5 puncture at day 14 following confirmation of paclitaxel-induced mechanical hypersensitivity. The rats were briefly anesthetized with 3% isoflurane and flexed over a tube and a 27-gauge needle inserted between the L5-S1 vertebrae, with a deflection of the tail indicating entry to the subarachnoid space. The dose of LPS-RS was chosen based on previously published studies, whereas the dose of MIP was based on the results of pilot studies wherein rats that received a dose of 100 μM MIP showed no effect on paclitaxel CIPN and rats injected with 1 mM MIP showed pronounced motor impairment. PBS (20 μL) and 500 μM MyD88 control peptide (CP, also in 20 μL PBS) were used separately as controls. To test whether LPS-RS may have an effect in preventing paclitaxel-induced CIPN, rats were treated with LPS-RS beginning 2 days before and then daily through day 2 after paclitaxel treatment. It was not possible to test the role of TRIF signaling in maintaining paclitaxel-induced neuropathic pain because there is no inhibitor available.

Mechanical Withdrawal Test

Mechanical withdrawal threshold was tested before, during, and following paclitaxel treatment by an experimenter (Y.L.) blinded to treatment groups during the mid-light hours (10 AM–2 PM). The 50% paw withdrawal threshold in response to a series of 8 von Frey hairs (.41–15.10 g) was examined by the up–down method, as described previously beginning with a filament with a bending force of 2.0 g.[19] Animals were placed under clear acrylic cages atop a wire mesh floor. The filaments were applied to the paw just below the pads with no acceleration at a force just sufficient to produce a bend and held for 6 to 8 s. A quick flick or full withdrawal was considered a response.

Rotarod Test

Rotarod performance was evaluated in the rats that were treated with intrathecal drugs to ensure a lack of treatment effects on motor function. Briefly, the rats were trained on the rotarod apparatus for 3 days prior to any intrathecal drug applications. Acceleration of the rotarod was set to increase from 0 to 40 r.p.m. over 5 minutes. Each rat was tested 3 times at 5-minute intervals, and the average of the latency to drop from the rod