Nonopioid Cannabinoid Type 2 Receptor Agonist (MDA7) Prevents Paclitaxel-Induced Central Sensitization and Mechanical Allodynia

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Introduction: Paclitaxel-induced neuropathy is associated with morphologic and biochemical alterations in dorsal root ganglia satellite cells, hyperplasia/hypertrophy of macrophages in the peripheral nervous system, and microglial and astrocyte activation within the spinal cord. This neuroinflammatory process causes central sensitization, leading to the persistent pain states. There is, therefore, a critical need to determine how paclitaxel alters pain sensation due to microglial activation and central sensitization. Cannabinoid receptor type 2 (CB2) is expressed primarily in the immune system including microglia in the CNS. MDA7 is a novel selective CB2 agonist that has shown efficacy in ameliorating microglial activation and preventing allodynia in rodent models of neuropathic pain. The central hypothesis is that paclitaxel triggers the expression of microglial P2X4 receptor in the dorsal horn. The influx of Ca^{2+} *via* P2X4 receptors enhances the activity of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) and transcriptional factor cyclic AMP response element binding protein (CREB), which subsequently increase ΔFosB expression and induces upregulation of microglial BDNF. The release of BDNF from microglia facilitates glutamatergic transmission and suppresses GABAergic transmission in dorsal horn neurons, contributing to paclitaxel-induced neuropathic pain. Since CB2 receptors are expressed on activated microglia, it is further hypothesized that the CB2 receptor functions in a negative-feedback loop and that early administration of a CB2 agonist can suppress the mechanism underlying the spinal microglial activation and BDNF upregulation, thus mitigating the central sensitization and pain behavior induced by paclitaxel.

Methods: Rats received 1.0 mg/kg i.p. of paclitaxel (or vehicle) daily for 4 consecutive days. MDA7 (15 mg/kg) or vehicle was injected (i.p.) 15 min before the administration of paclitaxel for 4 days and continued for another 10 days (a total of 14 days). AM630 (5 mg/kg i.p.), a selective CB2 receptor antagonist, was used in subsets of experiments. Mechanical sensitivity was assessed by using a series of Von Frey filaments with logarithmic incremental stiffness. Immunostaining and immunoblotting were performed to examine the activation state of microglia and the expression of BDNF and other proteins in dorsal horn (L4-5).

Results: Administration of paclitaxel resulted in tactile allodynia in rodent models (**Fig. 1**), which was alleviated by MDA7 treatment (**Fig. 1**). Because MDA7 is a specific CB2 agonist, it shows efficacy in treating mechanical allodynia in $CB2^{+/+}$, but not $CB2^{-/-}$ mice (**Fig. 1B,C**).

Paclitaxel significantly increased microglia activity (**Fig. 2A**) and CB2 expression (**Fig. 2B**) in the dorsal horn, which was attenuated by MDA7. Paclitaxel induced microglia polarization as shown by increased expression of IL-6 (M1 marker) (**Fig. 3A,B**) in the dorsal horn, which was attenuated by MDA7. In addition, MDA7 treatment was associated with increased expression of IL-10 (M2 marker) in the dorsal horn (**Fig 3. C**) and prevented the upregulation of microglial P2X4 in paclitaxel-treated rats (**Fig. 4**).

Paclitaxel also substantially increased the expression of CaMKIIα, phosphorylated CREB, ΔFosB and BDNF in the dorsal horn (L4-L5) tissue in rats treated with paclitaxel, which was reversed by MDA7 (**Fig. 5**). Furthermore, paclitaxel significantly increased the expression of glutamate receptor subunits NR2B and GluR1, and decreased expression of anion transporter KCC2 in the dorsal horn, which was reversed by MDA7 (**Fig. 6**).

Conclusion: Paclitaxel induced mechanical allodynia, microglial polarization, BDNF upregulation, and central sensitization in dorsal horn in the rats, which was prevented by MDA7. This study clarifies the spinal mechanism of paclitaxel-induced neuropathic pain and its modulation by a selective CB2 agonist, MDA7, which will ultimately lead to the development of effective approach to treat or prevent chemotherapy-induced neuropathy.

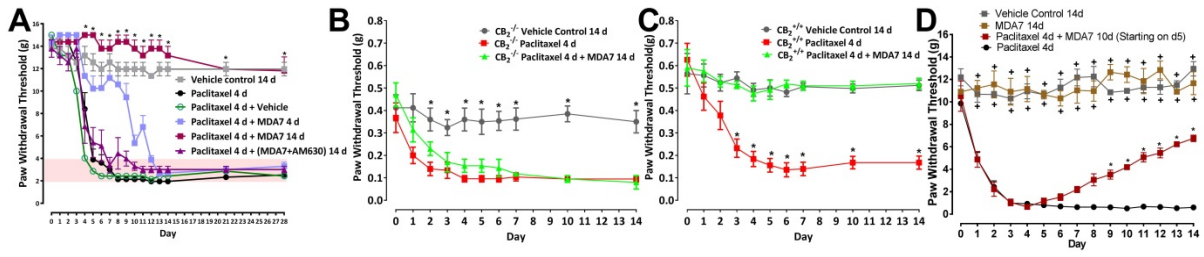

Fig. 1 MDA7 prevented paclitaxel-induced mechanical allodynia in rats (**A**) and $CB2^{+}/^{+}$ **(B)** but not $CB2^{-}/^{-}$ mice **(C)**. Significantly decreased paw withdrawal threshold was observed in the rats (n = 6 in all groups except vehicle control, n = 4) and $CB2^{+}/^{+}$ (n = 5), but not $CB2^{-}/^{-}$ (n = 5), mice injected with paclitaxel (1 mg/kg, day 1 to day 4). This was significantly extended by MDA7 (15 mg/kg, day 1 to day 14). **(D)** Treatment with MDA7 (15 mg/kg, day 5 to day 14) after the establishment of allodynia also significantly attenuated the mechanical hypersensitivity in the rats injected with paclitaxel (n = 6). $^{*,+}P < 0.01$ compared with other groups (linear mixed model with treatment and day effects with repeated measures over days).

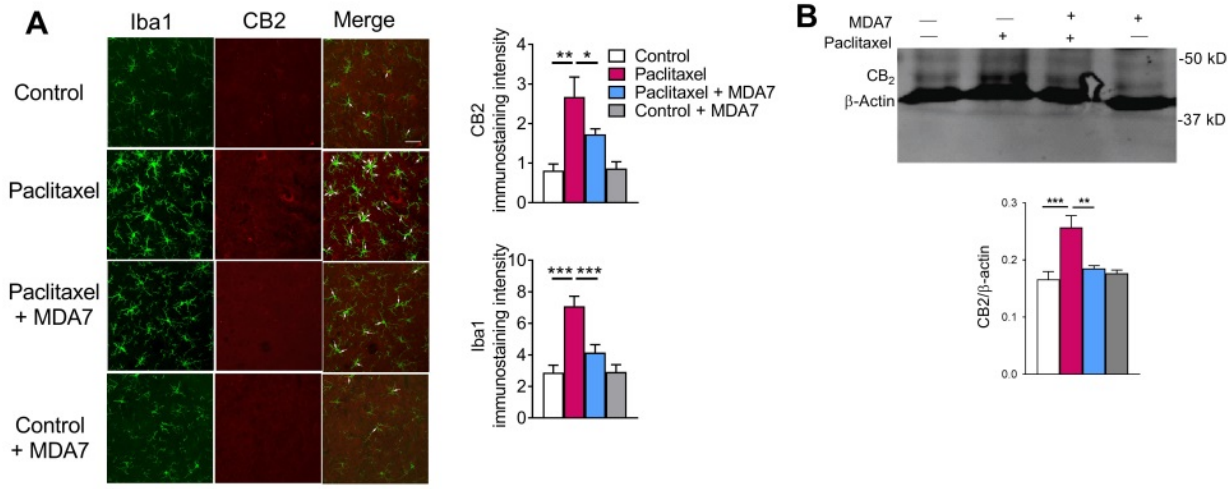

Fig. 2. Paclitaxel increased microglia activity and CB2 expression in the dorsal horn, which was attenuated by MDA7. (**A**) Increased microglia complexity and Iba1 (green) intensity was observed in dorsal horn in rats treated with paclitaxel, which was attenuated by MDA7. (**B**) The increased immunosignal of CB2 receptor in microglia was also observed in the dorsal horn of rats presented with allodynia after paclitaxel treatment. This effect was modulated by MDA7 (n = 6-7 per group). Scale Bar = 100 μm. *P<0.05; **P<0.01; ***P<0.001. Tissues were obtained on day 15 from animals described in Fig.1D.

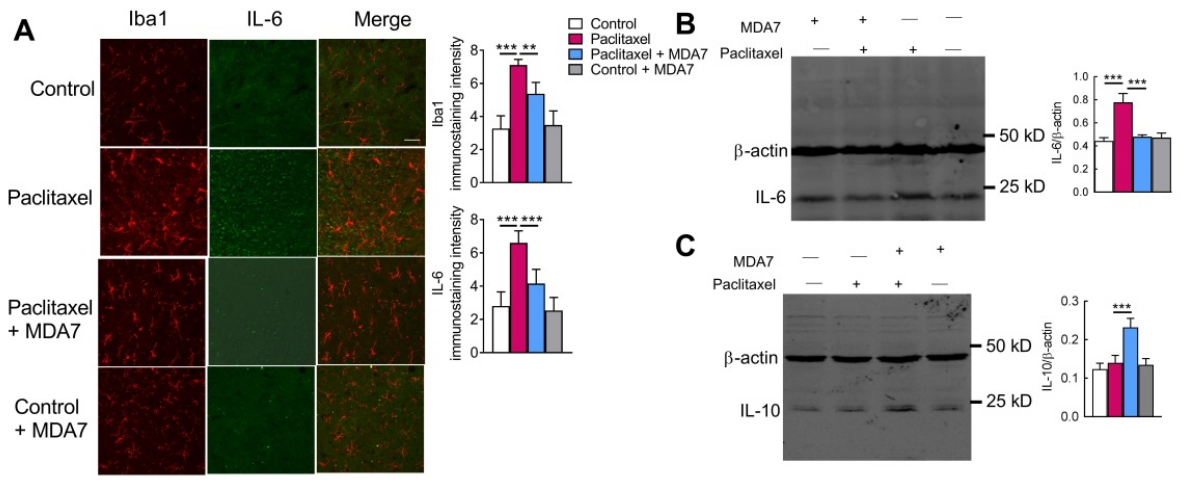

Fig. 3. Paclitaxel induced microglia polarization that was modulated by MDA7. (A) Increased expression of IL-6 (M1 marker) in microglia was observed in dorsal horn in rats treated with paclitaxel, which was attenuated by MDA7. (**B**) The increased immunosignal of IL-6 (M1 marker) in the microglia in the dorsal horn was attenuated by MDA7. (**C**) Treatment with MDA7 also increased the expression of IL-10 (M2 marker) in the dorsal horn of rats injected with paclitaxel (n = 6-7 per group). ***P<0.001. Scale Bar = 50μm.

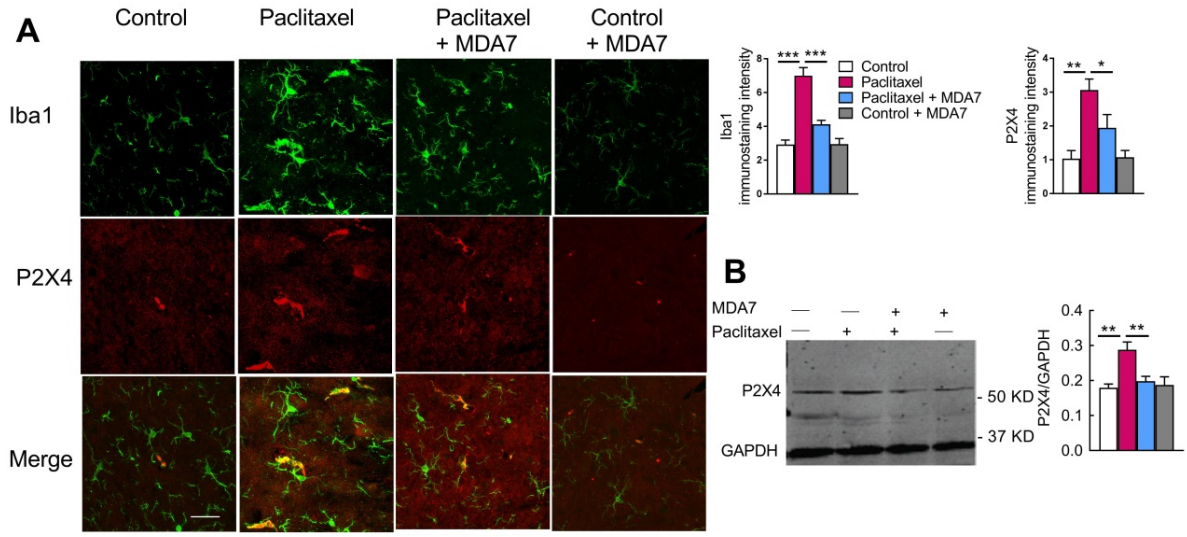

Fig. 4. Paclitaxel increased the expression of P2X4 in the microglia (A, n = 5) in the dorsal horn, which was attenuated by MDA7. (**B**) Immunoblotting study also found an increased P2X4 in dorsal horn, which was decreased by MDA7 (n = 6-7). Scale bar = 100 μm. *, P<0.05; **, P<0.01, ***, P<0.001.

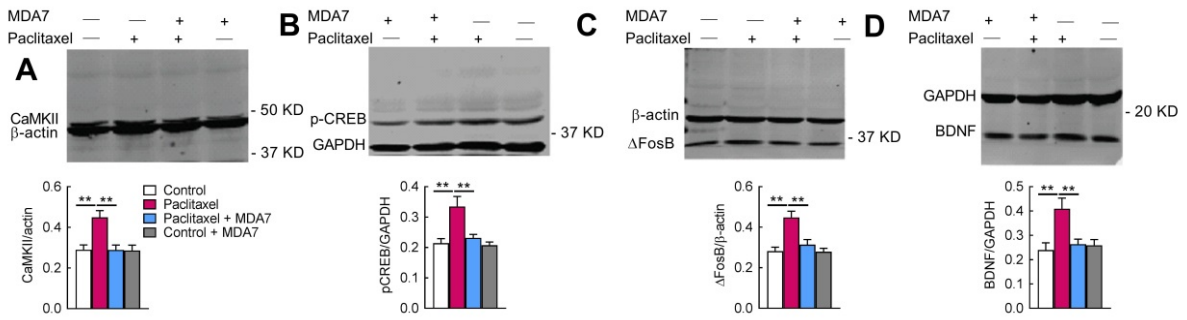

Fig. 5. Paclitaxel increased the expression of CaMKII (A), p-CREB (B), ΔFosB (C) and BDNF (D) in the dorsal horn in rats, which was attenuated by MDA7. **, P<0.01, n = 6-7 per group.

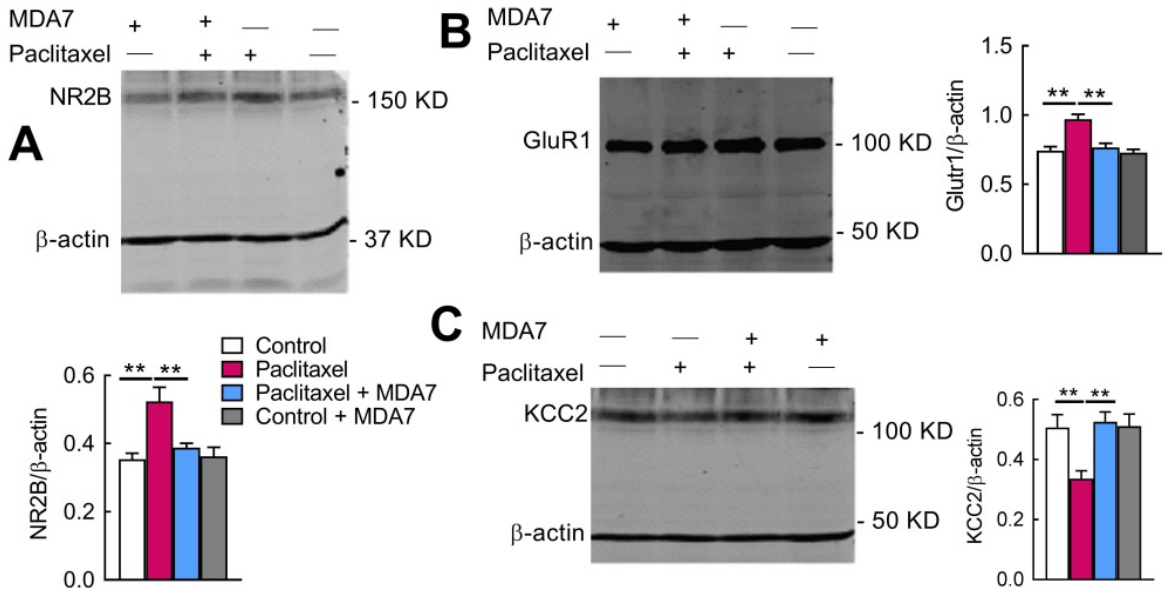

Fig. 6. Paclitaxel significantly increased the expression of glutamate receptor subunits NR2B (A) and GluR1 (B), and decreased expression of anion transporter KCC2 (C) in the dorsal horn in rats, which was reversed by MDA7. **, P<0.01, n = 6-7 per group.