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Intrathecal: Not Intravenous Administration of Evans Blue Reduces Pain Behavior in Neuropathic Rats

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Abstract : Introduction: Neuropathic pain induced by spinal or peripheral nerve injury is highly resistant to common painkillers, nerve blocks, and other pain management approaches. Recently, several new therapeutic drug candidates have been developed to control neuropathic pain. In this study, we used the spinal nerve L5 ligation (SNL) model to investigate the ability of intrathecal or intravenous Evans blue to decrease pain behavior and to study the relationship between Evans blue and the neural structure of pain transmission. Method: Neuropathic pain (allodynia) of the left hind paw was induced by unilateral SNL in Sprague-Dawley rats(n=10) in each group. Evans blue (5, 15, 50μg/10μl) or phosphate buffer saline(PBS,10μl) was injected intrathecally at 3days post-ligation or intravenously(1mg/200 μl) 3days and 5days post-ligation . Mechanical sensitivity was assessed using Von Frey filaments at 3 days post-ligation and at 2 hours, days 1, 2, 3, 5,7 after intrathecal Evans blue injection, and on days 2, 4, 7, and 11 at 14 days after intravenous injection. In the intrathecal group, microglia and glutaminergic neurons in the dorsal horn and VNUT(vesicular nucleotide transporter) in the dorsal root ganglia were tested to evaluate co-staining with Evans blue. The experimental procedures were performed in accordance with the animal care guideline of the Korean Academy of Medical Science(Animal ethic committee of Chungnam National University Hospital: CNUH-014-A0005-1). Results: Tight ligation of the L5 spinal nerve induced allodynia in the left hind paw 3 days post-ligation. Intrathecal Evans blue most significantly(P□0.001) alleviated allodynia at 2 days after intrathecal, but not an intravenous injection. Glutaminergic neurons in the dorsal horn and VNUT in the dorsal root ganglia were co-stained with Evans blue. On the other hand, microglia in the dorsal horn were partially co-stained with Evans blue. Conclusion: We confirmed that Evans blue might have an analgesic effect through the central nervous system, not another system in neuropathic pain of the SNL animal model. These results suggest Evans blue may be a potential new drug for the treatment of chronic pain. This research was supported by the National Research Foundation of Korea (NRF-2020R1A2C100757512), funded by the Ministry of Education.

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