Prenatal alcohol exposure enhances morphine-mediated toll like receptor (tlr)-4 actions and paradoxically prolongs nerve injury-induced pathological pain

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Morphine and MCC950 treatment while ongoing neuropathy prolongs allodynia in PAE mice

A. Spinal tactile

B. Contralateral paw

Figure 2: Morphine treatment prolongs the duration of allodynia only in the contralateral paw, suggesting that the prolonged allodynia results from the interaction between nerve damage, PAE and morphine.

Morphine interacts with PAE-primed immune cells via TLR4-NLRP3-IL-1β signaling pathway

Figure 3: Neuronal PAE mice were treated with Morphine or vehicle (saline). Treatment with NLRP3 inhibitor, MCC950, at D23-24 post-CCI showed pain-reversal effect as early as 1.5 hr post-injection (indicated with an asterisk). Data from a major CCI + Vehicle = MCC950 group (black circles) spontaneously recover from allodynia and suggest that MCC950 does not exert overt adverse effects on PAE mice.

Morphine and MCC950 treatment

Morphine and MCC950 treatment were given to naive-injured Sac and PAE mice starting from Day 14 post CCI, daily, for 5 subsequent days. The dose for MCC950 injection was 10 mg/kg.

In vitro stimulation of peripheral leukocytes

Peripheral leukocytes were isolated from the peritoneal cavity for morphine treatment. Cells were pre-treated with LPS (1 μg/ml) for 8 hours followed by incubation with Morphine sulfate (30 μM) in the presence of LPS or LPS + Morphine for another 20 hours. Media supernatant samples were stored at -80°C and cells were immediately processed for flow cytometric detection of caspase-1 protein activation. Supernatant samples were diluted 1:5 times to assess for mature IL-1β protein release using V-PLEX™ Immunoassay (MesoScale Discovery, MD2), according to manufacturer's instructions.

Statistical analysis

Repeated measures two-way ANOVA (Graph Pad Prism) was used for statistical analysis of the hind paw threshold responses. One-way ANOVA was applied for analyzing data from in vitro bone culture studies. Fisher's LSD test or Tukey's test was used for post hoc examination of possible group differences. *P < 0.05. Error bars represent SEM (standard error of mean).

Conclusions

Morphine may interact with the TLR4-NLRP3 actions in PAE-primed immune cells leading to exaggerated IL-1β production.

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