UNIVERSITÀ DEGLI STUDI DI PADOVA

PROJECT

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Project title: Role of Toll like receptor 4 in opioid-induced microglia activation and tolerance

Abstract (max 500 words)

Opioids are commonly prescribed for severe and chronic pain management. Unfortunately, chronic use of these drugs is limited by the development of tolerance, hyperalgesia, and risk of abuse. Moreover, it is now well-documented that opioids can initiate the innate immune cascade and the production of proinflammatory factors in the CNS, resulting in neuroinflammation. These events have been linked to suppression of analgesia and enhancement of opioid side effects.

In the CNS, the key cellular mediators of neuroinflammation are microglia, that are also protagonists as primary interlocutors for pain neurons. Protracted activation/alteration of microglia promotes persistent neuroinflammation that ultimately impacts neuron functionality. Thus, understanding the mechanisms underlying opioid-induced neuroinflammation, together with the identification of therapies based on the inhibition of neuroinflammation induced by opioids, is paramount to develop effective pharmacological strategies for pain management, minimizing the unwanted side effects of opioids.

With the aim to investigate the molecular mechanisms involved in opioid-induced neuroinflammation, this project will be focused on the study of opioid interaction with the receptor complex TLR4/MD-2. In our lab, this receptor has been widely studied both pharmacologically and by a molecular modeling approach, that allowed the identification of some small molecules able to inhibit microglia activation by binding the co-receptor MD-2. Initially, to verify the involvement of TLR4/MD-2 in opioid-induced neuroinflammation and to identify molecules able to suppress the opioid-induced activation of this receptor, we will exploit an *in vitro* approach based on primary microglia cultures exposed to different opioids. The inflammatory parameters will be examined using immunohistochemical staining, enzyme-linked immunosorbent assays and gene expression profiling. Then, thanks to the long-lasting collaboration with Prof. Moro's lab, the identified molecules will be evaluated by a combination of molecular docking studies and molecular dynamic simulations to reproduce their recognition process against both TLR4 and MD-2. Finally, results from this first phase will be advanced to the evaluation of suitable molecules to favor clinical outcomes in validated mouse models of opioid-induced tolerance by measuring the response to thermal and mechanical noxious stimuli, as well as the effect on *in vivo* inhibition of neuroinflammation and microglia activation.

Collectively, this project will provide the pharmacological basis for developing TLR4/MD-2 inhibitors as potential drug candidates to reduce tolerance and enhance clinical utility of opioids in the chronic pain management.