UNIVERSITÀ DEGLI STUDI DI PADOVA

PROJECT

Tutor's Name	Prof.ssa Morena Zusso
Cotutor's Name	

1) Project title: Pharmacological characterization of Toll-like receptor 4 as a target for opioids in microglia.

2) Abstract (max 500 words)

Chronic pain is a major source of suffering and represents an important health and social problem that affects an estimated 20% of the world's population. Opioids are the most widely used class of analgesics for acute and chronic pain conditions. Unfortunately, chronic use of these drugs is limited by the development of analgesic tolerance, opioid-induced 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. Protracted activation/alteration of these cells promotes persistent neuroinflammation that ultimately impacts neuronal transmission and plasticity.

Given the central role of Toll-like receptor 4 (TLR4) in the regulation of signaling events in innate immune cells, this receptor has been proposed as an off-target site for opioid action.

To investigate the molecular mechanisms involved in opioid-induced neuroinflammation, this project aims at validating TLR4 as an opioid target for future development of effective and safe pharmacological agents for pain management. 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, we will evaluate the direct binding of several clinically used opioids and endogenous opioid peptides to the TLR4/MD-2 complex using surface plasmon resonance and oligomerization assays. Next, the functional effects of selected opioids and related mechanisms will be studied in microglia cells that naturally express TLR4 (i.e., primary cultures of rodent microglia, human iPSC-derived microglia). The inflammatory parameters will be examined using immunohistochemical staining, enzyme-linked immunosorbent assays and gene expression profiling. Then, 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, we will investigate whether inhibitors of LPS-induced TLR4 activation, previously identified by our group and others, affect the interaction of opioids with TLR4/MD-2 complex and/or its downstream signaling.

Results from this first phase will then 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.