Sorbonne Université/China Scholarship Council program 2021

Thesis proposal

Title of the research project: Alleviating corneal pain with opioids

Keywords: ocular pain, dry eye, opioid receptor, inflammation

Joint supervision: no

Joint PhD (cotutelle): no

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SORBONNE UNIVERSITÉ

Subject description (2 pages max):

1) Study context

Corneal neuropathic pain (CNP) is a chronic condition associated with symptoms such as irritation, dryness, burning, aching, and light sensitivity (1). This condition is increasingly recognized in patients with chronic dry eye (DE) syndrome (2,6,7) and dramatically affect the quality of life of patients who frequently develop associated anxiety and depression

Currently, our knowledge and appreciation of the pathophysiology of CNP is in its early stages, and therapeutic treatment is currently satisfactory (5,6). CNP is triggered by corneal nerve terminals which cell bodies are located in the trigeminal ganglion (TG) and sending their central axons into the trigeminal brainstem sensory complex. The field of pain needs a better characterization of this CNP network (from the cornea towards brain areas), but also needs to identify novel therapeutic target for alleviating this painful condition. We actively contributed in deciphering part of these mechanisms triggering peripheral and central sensitization mechanisms during the last decade (3,4,7-9). We developed a clinically-relevant mouse model of chronic corneal pain associated with DE. This model is characterized by morphological and functional corneal nerve abnormalities (3,4,7). We found an increased neuronal activation (Fos expression) in TG and TBSC, as well as a presynaptic plasticity in CNP mice three weeks after the surgery (3,4). Moreover, we highlighted that CNP mice exhibited higher expression and activation of macrophages and microglia in the TG and TBSC, respectively. Moving forward, we fund that CNP mice developed anxiety, which is consistent with clinical data.

More recently we have oriented our research on the opioid system as a possibility for ocular pain research and therapy (8,10,11) since this area of research has been poorly investigated yet. Pain can be modulated by exogenous and endogenous opioid peptides, which bind to mu (MOR), delta (DOR) and kappa (KOR) receptors. Targeting peripheral opioid receptors became an area of renewed interest. We found antinociceptive and anti-inflammatory properties of a dual inhibitor of the enkephalin-degrading enzymes topically administered (11) and that repeated topical ocular administration of DAMGO, a MOR-selective ligand, markedly reduced the mechanical corneal hypersensitivity (8) in a model of acute pain. Despite the current evidence for the potent analgesic effects of topical MOR agonist in acute inflammatory ocular pain model, more research is needed to evaluate their use in chronic corneal pain model.

The general objectives of this project are **1**- the identification of the corneal nociceptor classes expressing MOR under basal and chronic dry eye conditions, **2**- the effects of topical MOR agonist on the responsiveness of inflammatory-related gene expression, corneal hypersensitivity and anxiety-like behaviors related to CNP, **3**-Identification of a functional CNP network and potential changes induced by MOR activation.

2) Details of the proposal

Work Package 1: Identification of the subpopulation of corneal neurons expressing MOR under basal and persistent dry eye conditions. This WP aims at identifying the subpopulation of corneal neurons expressing MOR. To do this, neuronal retrograde tracer will be placed on the cornea to label corneal neurons in the TG. Co-expression of MOR with markers of polymodal (TRPV1), cold-sensing neurons (TRPM8) and mechano (Piezo2) sensory neurons will be performed by *in situ* hybridization and immunohistochemistry in sham (control) and CNP mice at D21. Tissue sections will be imaged by Nanozoomer (digital slide scanner) and quantified with ImageJ.

Work Package 2: Effects of topical MOR agonist on corneal nociception, neuropathic and inflammatory-related genes, neuronal activation in TG. This WP has two main outcomes: **1-** We aim to extend our knowledge about the molecular signature (gene expression) of the TG and brain by using predesigned 96-well (BioRad) including gene related to pain and inflammation (opioid receptors, TRPV1, P2X4R, IL1, IL6, voltage gated ion channels) (4). To assess this, TG and brain from sham and CNP mice topically treated or not with a MOR agonist (twice a day, for 3 weeks) will be analyzed at D0 and D21. This

task will allow to identify which of the 96 genes are modulated in our model as well as their possible modulation by topical MOR agonist. **2**- Effects of topical MOR agonist in chronic corneal pain. Topical treatment with a MOR agonist will be evaluated on relief of ongoing (spontaneous) pain and on the aversiveness of mechanical and chemical stimulation as previously described in (3,4). The spontaneous pain and mechanical/chemical hypersensitivity will be studied at D0 and every week, 15 minutes after the last topical administration of PBS, MOR agonist and MOR agonist + naloxone methiodide (MOR antagonist).

Work Package 3: Does topical MOR agonist reduced anxiety-like behaviors associated with chronic corneal pain? We recently found that mice with chronic pain develop anxiety-like behaviors at D21.We thus make the hypothesis that repeated administration of topical MOR agonist -besides inhibiting nociceptive responses- will also affect the anxiety -like behaviors associated with persistent DED pain. In this set of experiments, the elevated plus-maze and light-dark box tests will be used at D21. All tests are routinely used in the team.

Work Package 4: Building of a functional CNP network with *c-fos* expression. Brain-wide map of the activity-regulated gene *c-fos* is a powerful approach to generate a functional network associated with a pathological condition. This task aims at identifying interregional correlations of brain regions that are activated by CNP (corneal pain connectome). Histological sections from TG and brain from sham and CNP mice will be immunostained with Fos (to monitor neuronal activation) Next, we will evaluate potential disruption of the functional CNP network after MOR activation. Here, we want to assess the impact of MOR activation on previously identified interregional correlations of brain regions that are activated by CNP.

3) References

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4°) Profile of the Applicant (skills/diploma…)

We will consider candidates with a Master degree in Physiology, Neuroscience, or a related field. The ideal candidate has skills in animal behavior, cellular and molecular biology, and good communication skills and a team-oriented work style.

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