PROJECT 1

DoS: Giulio Ferrari

Title: "The role of neuroinflammation and its inhibition in ocular surface pain: pre-clinical models and clinical implications"

Curriculum: Experimental and clinical medicine

Link to OSR/UniSR personal page:
https://research.hsr.it/en/divisions/neuroscience/eye-repair-lab/giulio-ferrari.html

Project description (*Number of characters, including spaces*: 2.000 - 3.000):
Corneal nerve injury is a consequence of highly prevalent human diseases, such as mechanical/chemical trauma, infections and surgeries. Nerve disruption triggers a complex process named neuroinflammation which ultimately results in pain, recruitment and activation of leukocytes. Mechanisms controlling neuroinflammation are still under scrutiny; however, a role for pro-inflammatory neurotransmitters, including Substance P (SP), Calcitonin Gene Related Peptide (CGRP) and noradrenaline (NA) has been suggested.
Pain induced by neuroinflammation has critical clinical implications, as it affects millions of patients worldwide and is associated with significant reduction in the quality of life.
In particular, SP is released from sensory nerve endings and exerts its actions by binding to the Neurokinin-1 Receptor (NK-1R), localized on nerves, immune and epithelial cells. Previous studies have demonstrated that in vivo inhibition of SP activity, either through its ablation or topical administration of selective NK-1R antagonists, leads to decreased ocular inflammation and reduces corneal opacity, perforation rate, and angiogenesis. Preliminary data from a compassionate clinical study suggest that SP blockade may result in ocular analgesia. However, the role of SP as a modulator of pain on the ocular surface is still unclear.
This project aims to investigate the role of SP and NK-1R in the generation of ocular pain. To this end, we will use a well-established pre-clinical model of ocular surface pain. In order to assess the relevance of SP/NK-1R pathway, we will employ a SP-KO mouse, the B6.Cg-Tac1tm1Bbm/J (TAC1-KO), where the Tac 1 gene -responsible for the production of the tachykinin SP-has been deleted.
In addition, we will test the effect of pharmacological blockade of SP activities by using selective NK-1R antagonist aprepitant. Aprepitant has the additional advantage of being clinically approved, which can further accelerate clinical translation. Different aprepitant concentrations and formulations will be tested. Additional groups of non-treated, or vehicle-treated mice, will be used. Finally, two groups of mice will be treated with Diclofenac and Oxybuprocaine (positive controls). At the end of the experiment, the eyes will be harvested to evaluate nerve morphology. In addition, SP, NK-1R, CGRP, NA, and inflammatory cytokines will be quantified in the cornea by ELISA, Western Blot, and PCR.
To dissect the mechanism(s) inducing analgesia, we will harvest the trigeminal ganglia and measure the expression of key pain-related markers including SP, CGRP, and IL-6 by means of proteomic analysis.
To assess the clinical relevance of our findings we will run -in conjunction with the Cornea Unit- an observational clinical trial in a cohort of patients affected by ocular pain. Tear fluid samples will be collected: (i) to quantify SP, CGRP, NA, and inflammatory cytokines and (ii) to correlate their expression with pain-associated clinical surrogate markers.

Skills to be acquired by the student:

Animal model induction/handling

PCR, ELISA, Western blot techniques

Immunofluorescence and Immunohistochemistry techniques

Bright field, Confocal, Epifluorescence microscopy

Basic statistical analysis knowledge

References (max. 3)

- Bignami, F., Rama, P., Ferrari, G., 2016. Substance P and its Inhibition in Ocular Inflammation. Current Drug Targets 17, 1265–1274.

- Marco, B., Alessandro, R., Philippe, F., Fabio, B., Paolo, R., Giulio, F., 2018. The Effect of Aging on Nerve Morphology and Substance P Expression in Mouse and Human Corneas. Invest Ophthalmol Vis Sci 59, 5329–5335. https://doi.org/10.1167/iovs.18-24707
- Rosenthal, P., Borsook, D., 2016. Ocular neuropathic pain. Br J Ophthalmol 100, 128–134. https://doi.org/10.1136/bjophthalmol-2014-306280