# PURPLE CORN EXTRACT AS ADJUVANT THERAPY FOR THE PREVENTION AND TREATMENT OF TRIGEMINAL PAIN: ROLE OF MICROGLIA AND OF THE GUT MICROBIOTA

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**Introduction:** Trigeminal (TG) pain is a debilitating condition whose pharmacological treatment still represents an unmet medical need. In the search of new approaches, the pivotal role of a correct diet in promoting health is clearly emerging beyond drugs. Studies performed with different sources of anthocyanins (ACNs) showed that they can protect against several inflammation-related diseases (Tsuda, Mol Nutr Food Res 2012), but very few data are available on pain syndromes, with no hints on TG pain. Additionally, accumulating evidence introduces the concept of "gut-brain axis" as a bidirectional signaling between the gut microbiota and the central nervous system (CNS), in both physiology and pathology. With the present project we tested, for the first time, the effects of an ACN-rich dietary supplement in an *in vivo* model of TG sensitization, in combination with a known analgesic, i.e. acetylsalicylic acid (ASA). Moreover, we elucidated the role of microglial cells in the CNS and infiltrating macrophages in the PNS in the development of orofacial pain and their modulation by ACN-enriched dietary supplement. Finally, we investigated the effect of ACNs on the modulation of specific components of the gut microbiota with known effect on brain inflammation.

**Methods:** We utilized isogenic maize model foods: purple corn with increased ACN content, and yellow corn without ACNs as control, both provided as water-soluble granules (Petroni et al., Planta 2014). TG sensitization was induced *in vivo* by the unilateral injection of Complete Freund's Adjuvant (CFA) in the temporomandibular joint of male rats. Orofacial mechanical allodynia was assessed by Von Frey test (Magni et al., Glia 2015). The expression of the microglia/macrophages marker Iba1was evaluated by immunohistochemistry. For the analysis of microbiota composition, the bacterial taxonomic profile was reconstructed from fecal samples of animals before and after administration of either purple and yellow corn supplements by means of 16S rRNA profiling protocol (Milani et al., PLoS One 2013).

**Results:** Animals receiving water and yellow corn developed ipsilateral orofacial allodynia, which was instead significantly reduced in rats that received purple corn. The effect of purple corn supplement was fully comparable to the anti-allodynic action exerted by ASA, and purple corn extract was as effective as ASA in inhibiting the TG infiltration of $Iba1^+$ macrophages in CFA-injected rats. Conversely, purple corn alone significantly reduced microglial activation in the brainstem, with no effect exerted by ASA. Moreover, the treatment of LPS-activated microglia in vitro with purple corn extract reduced the production of pro-inflammatory mediators and promoted a shift towards an anti-inflammatory phenotype. Finally, purple corn administration significantly modified the gut microbiota toward an anti-inflammatory taxonomic profile.

**Conclusions:** Our results demonstrate that the administration of ACN-enriched purple corn extract has a protective effect on the development and maintenance of orofacial allodynia in an *in vivo* model of inflammatory TG pain, reduces TG macrophage infiltration, and microglial activation both *in vivo* and in vitro. The protective effect of purple corn is comparable to the anti-inflammatory effects of ASA, which however does not modify microglia activation. Analysis of microbiota composition indicates that purple corn promotes the gut colonization by anti-inflammatory taxa. We therefore speculate that purple corn extract acts to prevent inflammatory pain through different cellular/molecular mechanisms that could also involve the gut-brain axis. Therefore, we foresee a possible application of ACN-rich dietary supplements as co-adjuvant to pharmacological treatments or as new preventive strategy against TG pain, aimed at reducing drug dosage and side effects and improving patients' compliance to therapy.

Results are published in Magni et al., Front Cell Neurosci 2018, 12:378.