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Strokes and traumatic spinal cord and brain injury

MULTIDISCIPLINARY FOCUS FOR MODULATION OF NEUROIMMUNE CROSSTALK ASSOCIATED WITH PATHOLOGICAL PAIN AFTER CHRONIC LESION OF THE SPINAL CORD

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1. Project Summary

Central neuropathic pain (CNP) following spinal cord injury (SCI) is developed in more than half of patients and around one third of those report the pain to be severe. CNP after SCI has been associated with impairments in a variety of areas, including physical functioning and mobility, mental and cognitive activities, social interaction and community reintegration, sleep, employment, and quality of life. Moreover, people who experienced pain after SCI showed poorer health, lower satisfaction with life and greater risk of depression. To address this health concern, several pharmacological treatments have been used to alleviate CNP. However, current treatments are often ineffective because they target only one or two of the several mechanisms that comprise CNP. Nowadays, to design a personalized therapeutic approach, treatment consists in a trial-and-error of different strategies, including antidepressants, antiepileptics, GABA-antagonists, local anaesthetics, NMDA-antagonists, cannabinoids and opioids. However, only one third of patients respond to pharmacological treatments when compared with placebo and the best pharmacological strategy results in a reduction of only 20–30% in pain intensity, frequently accompanied by severe side effects.

Given the lack of effective treatments, it is necessary to develop new pharmacological strategies not only for neuropathic pain relief but also to prevent its chronification. Among the potential pharmacological strategies aimed at modulating pathological pain the use of polyphenols could be highlighted, since preclinical evidence of their antinociceptive effects can be found in the scientific literature. The properties attributed to polyphenols that may explain pathological pain modulation are free radical scavenging/antioxidant, immunomodulatory, neuroprotective, anti-apoptotic and autophagy-regulating activities. However, although several studies have been specifically aimed at elucidating the effects of polyphenolic treatments on neuropathic pain development, most of them have been conducted in preclinical models unrelated to SCI, such as peripheral neuropathic pain, diabetic neuropathic pain, or alcoholic neuropathy. Furthermore, although studies on polyphenol treatment after SCI are available, most of them have focused on motor recovery or spinal cord regeneration, leading to a lack of information despite promising results on their effects on modulating pathophysiological processes that may also be related to neuropathic pain development. In this context, the main objective of this project was to study the

preventive and analgesic effects of two polyphenolic plant extracts (GSE and CE) in SCI-induced CNP development in mice, and to compare them with the effects of epigallocatechin-3-gallate (EGCG), one of the most studied polyphenols in the field of neuropathic pain. The extracts were obtained from plant sources using saline solution as vehicle so that their administration was physiologically compatible.

After SCI, the effects of repeated EGCG, GSE and CE treatments on neuropathic pain behaviours (hyperalgesia and allodynia) were evaluated during the acute, intermediate, and chronic phases of injury. In addition, gliosis and the expression central sensitisation-related biomarkers were analysed in both spinal cord and pain-processing involved supraspinal structures. In all the experiments, the pharmacological safety was also evaluated to ensure the absence of toxicity of the treatments.

On the other hand, in addition to the problem of the lack of suitable treatments, there are also no specific biomarkers of neuropathic pain that would facilitate its diagnosis in the field of pain medicine. Currently, pathological pain diagnosis is mainly based on clinical criteria that are often extended over time. Consequently, the pain continues to become chronic which in turn triggers the appearance of comorbid psychological diseases such as anxiety and depression that drastically affect the quality of life of patients. In this context, this project was also aimed at developing new strategies with potential diagnostic use for neuropathic pain. Considering that pathological pain is a complex disease, the use of classic laboratory techniques such as immunohistochemistry or molecular biology tests were not the best option, so we used analytical chemistry techniques combined with chemometrics and artificial intelligence to study complete molecular profiles as potential biomarkers. To this end, acute and chronic central neuropathic pain was induced by spinal cord injury in mice, the sera of these mice were analysed by mass spectrometry and the resulting data was subsequently processed with artificial neural networks. In addition, this methodology was also used to analyse samples of nervous tissue, in this case not for diagnostic use but to lay the foundations for the characterization of potential therapeutic targets present in that tissue.

2. Results obtained

Both preventive GSE and CE administration during the first week post-SCI, resulted in the attenuation of mechanical allodynia and thermal hyperalgesia development during the acute phase of SCI without either weight-loss or increase of serum biomarkers hepatotoxicity or nephrotoxicity, in contrast to EGCG treatment that showed signs of potential systemic toxicity. Antinociceptive effects of polyphenolic extracts were associated with the prevention of both gliosis and upregulation of algogens related to central sensitization and neuron-glia crosstalking in the spinal cord. In addition, GSE and CE treatments modulated these pathophysiological processes in pain-modulation related supraspinal structures such as anterior cingulate cortex (ACC) and periaqueductal gray (PAG). On the other hand, while repeated administration of EGCG during the third week post SCI modulated neither mechanical allodynia nor thermal hyperalgesia induced by SCI during the intermediate phase of injury, the same administration pattern for both GSE and CE modulated these reflexive pain responses with no associated systemic toxicity. In addition, CE administration modulated depressive-like behaviour detected in spinal cord injured mice at intermediate phase of injury. Finally, repeated administration of GSE and CE during the first-, third- and sixth-week post SCI prevented thermal hyperalgesia and mechanical allodynia development up to the chronic phase of injury, with no associated systemic toxicity. In addition, GSE and CE treatments also modulated both affective-motivational disturbances (anhedonia, depression and anxiety) and social interaction impairment developed in chronic SCI-animals. Antinociceptive effects of GSE and CE were associated not only with the modulation of spinal cord microgliosis but also with the modulation of astrogliosis and neuron-glia crosstalk signalling in the supraspinal structures ACC, amygdala, dorsal and ventral PAG and rostral ventromedial medulla (RVM) as well as microgliosis in ventral PAG. Overall, the results suggest that a mixture of polyphenols present in natural extracts may be a suitable pharmacological strategy to either prevent or attenuate the development SCI-induced neuropathic pain by modulating not only the reflexive pain responses (more related to the sensory-discriminative dimension of pain) but also the non-reflexive pain responses (included in the affective-motivational dimension of pain). These compounds exert their effects not only at the site of injury by modulating gliosis and the expression of central sensitisation-related biomarkers, but also on supraspinal structures closely related to expression and modulation of central neuropathic pain.

3. Relevance to future potential implications

Regarding the treatment with the obtained extracts, the results of the project suggest that the mixture of polyphenols present in the natural extracts may be a suitable pharmacological strategy to prevent or attenuate the development of neuropathic pain induced by spinal cord injuries, not only reflex evoked pain responses but also non-reflexive pain responses expressed as emotional and social interaction disturbances. This is so because these compounds exert their effects not only at the site of injury by modulating gliosis and the expression of central sensitization-related biomarkers in the spinal cord, but also on supraspinal structures associated with the expression and modulation of central neuropathic pain. It is worth mentioning also that these extracts have been developed using physiological saline solution compatible with organisms and demonstrating the absence of systemic toxicity.

Regarding the results of molecular profiles, as mentioned above, the results provide a promising tool not only for the diagnosis and monitoring of neuropathic pain derived from spinal cord injury in both the acute and chronic phases, but also to determine specific biomarkers and possible therapeutic targets useful for designing new pharmacological strategies in the near future.

4. Generated scientific bibliography

This project has allowed the realization of two doctoral theses:

Dr Meritxell Deulofeu Figueras. Doctoral thesis University of Girona, *Cum Laude* with international mention (December 2019).

Dr Anna Bagó Mas. Doctoral thesis University of Girona, *Cum Laude* with international mention (April 2022).

As regards scientific publications, one manuscript is under review and three more are being written. Publications will be announced soon.