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SPINAL AND SUPRASPINAL CHARACTERIZATION OF AN INFLAMMATORY CFA-INDUCED MODEL OF VULVODYNIA

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OBJECTIVE: Vulvodynia is a gynecological disease characterized by chronic pain in the vulva and tissues surrounding access to the vagina. The chronic vulvar pain that characterizes the disorder is typically associated with severe burning, dyspareunia (pain during sexual intercourse) and redness. Factors such as genetic predisposition, hormonal imbalances, pelvic floor dysfunction, and immune system dysregulation have been suggested to play a role in its establishment, rising the need of further mechanistic understanding and search for new therapeutical targets.
METHODS: In this study we used female C57BL/6J mice in which vulvodynia was induced by one injection of Complete Freund Adjuvant for up to four weeks. During these 28 days behavioral, electrophysiological and immunohistochemical analysis were performed. We employed pharmacological interventions using GABApentin, amitriptyline, and PeaPol, a combination of palmitoylethanolamide and polydatin.
RESULTS: All three drugs showed efficacy in alleviating tactile allodynia and depressive-like behavior. Concurrently, we also observed a normalization of the altered neuronal firing and a reduction of microglia hypertrophic phenotypes.
CONCLUSIONS: Our results suggest a role for microglia in neuroinflammatory phenomena surrounding spinal neuronal overexcitability in vestibulodynia, emerging as a novel therapeutic target. Treatment with PEAPol improved not only evoked pain, but also spontaneous pain, promoting mice well-being, as observed in nesting behavior. Finally, for the first time in CFA-induced vestibulodynia model, we showed the development of depressive-like symptoms, often present also in patients, reinforcing the translational value of the study.

N-PALMITOYLETHANOLAMIDE-OXAZOLINE (PEA-Oxa) REDUCES SEIZURE SEVERITY AND RELATED NEUROPSYCHIATRIC-LIKE COMORBIDITIES IN THE PENTYLENETETRAZOL KINDLING MODEL

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OBJECTIVE: Antiseizure medications (ASMs) are the first-line drugs to treat epilepsy. Given the significant adverse reactions of ASMs, the search for safer molecules is shifting towards bioactive compounds such as N-acylethanolamines. N-palmitoylethanolamide-oxazoline (PEA-Oxa) has attracted considerable interest due to its neuroprotective and anti-neuroinflammatory properties. This work aimed to study the effects of different doses of PEA-Oxa in Pentylenetetrazol (PTZ)-induced kindling, and its effects in modulating neuroinflammation.
METHODS: A sub-convulsant PTZ dose (30 mg/kg i.p.) was administered to C57/BL6J (B6) mice every other day up to kindling development. PTZ-kindled B6 mice received chronic PEA-Oxa treatment at different doses. All groups were subjected to several behavioral tests (passive avoidance, novel object recognition test, three-chamber tests, elevated plus maze, and forced swimming test) for assessing neuropsychiatric-like deficits. Finally, different neuroinflammatory markers were evaluated.
RESULTS: PTZ-kindled B6 mice showed an increased seizure severity score and reduced behavioral performance, such as cognitive impairment, sociability, and anxiety. PEA-Oxa treatment reduced the seizure severity score and rescue behavioral performance in B6 mice. We also revealed that PEA-Oxa has anti-inflammatory effects by reducing several neuroinflammatory markers dose-dependently.
CONCLUSIONS: The present data supports the beneficial effects of PEA-Oxa supplementation against seizure and related neuropsychiatric comorbidities in the PTZ-Kindling model. Further experiments are needed to clarify the mechanisms by which PEA-Oxa exerts beneficial effects in PTZ-induced kindling.

PUDENDAL NERVE CONSTRICTIONS (PNC) AS A NEW ANIMAL MODEL OF VULVODYNIA

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OBJECTIVE: Vulvodynia is a gynecological condition characterized by persistent discomfort in the vulvar region. This enduring vulvar pain is often accompanied by intense burning sensations, dyspareunia (pain during intercourse), and erythema (redness). Current *in vivo* experimental models of vulvodynia, such as CFA-induced vulvodynia, only partially represent the entire affected population. Pudendal nerve entrapment is one of the most common causes identified to date. Therefore, in our study, we developed a novel *in vivo* model of vulvodynia by inducing constriction of the pudendal nerve.
METHODS: Female C57BL/6J mice, via surgical procedures, has been tied monolateral the pudendal nerve using surgical silk thread. The vulvar mechanical allodynia (Von Frey test) and the general welfare (Nesting test) were measured from week 1 to 3 post pudendal nerve constrictions (PNC), during these weeks the animals were orally treated with Gabapentin and Amitriptyline. From 21st day immunofluorescence and electrophysiology analysis were performed.
RESULTS: Surgery reduced the vulvar withdrawal threshold in mice from 1 to 3 weeks post-PNC induction, with treatment reducing allodynia by week 3. Nesting behavior worsened after PNC but improved with treatment. At 2 weeks, the tail suspension test revealed depressive-like behavior, which was alleviated by drugs at 3 weeks. By 21 days, reactive microglia increased in the ipsilateral spinal cord, and both ongoing and evoked nociceptive neuron activity also rose.
CONCLUSIONS: In conclusion, future behavioral and immunofluorescence studies will be conducted to further characterize this new model but, to date, our findings underscore the utility of the PNC model in elucidating the mechanisms of vulvodynia disease and highlight its potential as a valuable tool for future preclinical research and drug discovery efforts.

POOLED ANALYSIS DEMONSTRATED EFFICACY OF THE TREATMENT OF COMBINATION OF ANTI-CGRP(R) mAbs AND ONABOTULINUMTOXIN A IN CHRONIC MIGRAINE

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OBJECTIVE: Chronic migraine is a neurovascular disorder, that remarkably impairs the patients' quality of life, especially in refractory 40% cases despite the novel monoclonal antibodies (mAbs) directed towards the signaling of the calcitonin gene-related peptide (CGRP). Onabotulinumtoxin A was demonstrated to be effective and safer than topiramate. Therefore, the objectives of this study were to: 1) assess the real-world evidence of efficacy of anti-CGRP mAbs in resistant chronic migraine; 2) design a clinical trial to assess efficacy and safety of combination therapy in refractory chronic migraine.
METHODS: The retrospective phase included a wide sample of 298,000 inhabitants, 213,000 under 60 years of age. The clinical trial to evaluate efficacy and safety of the combination treatment followed SPIRIT and CONSORT statements to recruit patients suffering from chronic migraine and refractory to the most common preventative treatments. The results were statistically evaluated for differences using $\chi 2$ test considering $p < 0.05$ significant and a prespecified statistical analysis plan for the combination therapy clinical trial was set.
RESULTS: Pooled analysis demonstrated that treatment of combination of anti-CGRP(R) mAbs and onabotulinumtoxin A provides ≥50% reduction of monthly headache days (MHDs) in up to 58.8% of patients *vs*. onabotulinumtoxin A alone. According to results, early diagnosis of migraine improves therapeutic outcomes with mAbs, that reduced MHDs, MMDs and NRS score. Interestingly, also patients arriving to clinical observation aged over 50 were detected. The outcome measures of the trial will include reduction of monthly migraine days (MMDs) after 1, 3 and 6 months of treatment with mAbs and of pain intensity assessed by the numeric rating scale (NRS) after 6 months of treatment.
CONCLUSIONS: The synergic/additive mechanism of the combination may be due to inhibition of CGRP release from thin unmyelinated C fibers dural nociceptors and prevention of CGRP action.