

PROBIOTIC SUPPLEMENTATION ALLEVIATES MIGRAINE-LIKE PAIN BY RESTORING INTESTINAL BARRIER INTEGRITY AND ENDOCANNABINOID HOMEOSTASIS

S. Facchetti[1|2], C. Demartini[1], M. Francavilla[1], B. Marfella[3], L. Cristino[3], R. Greco[1], C. Tassorelli[1|2]

[1]Section of Translational Neurovascular Research, IRCCS Mondino Foundation, Pavia, Italy; [2]Department of Brain and Behavioral Sciences, University of Pavia, Italy; [3]Istituto di Chimica Biomolecolare del CNR, Pozzuoli (NA), Italy

Background. Gut microbiota and the endocannabinoid system (ECs) play a key role in regulating body homeostasis and nociceptive information. Intestinal dysbiosis can compromise the gut barrier integrity, leading to a systemic pro-inflammatory response. We previously observed an increase in serum lipopolysaccharide-binding protein levels, indicative of intestinal barrier impairment and alterations in gut microbiota composition in the nitroglycerin (NTG)-induced animal model of chronic migraine. The present study aimed to investigate whether probiotic-mediated restoration of gut microbiota attenuates migraine-like pain by improving intestinal barrier integrity and modulating ECs signalling.

Methods. Adult male Sprague-Dawley rats were treated with NTG (5 mg/kg i.p.) or vehicle every other day for nine days (5 injections). A subgroup of NTG animals received a multistrain probiotic in their drinking water for the entire period duration, whereas rat without the probiotic treatment were provided with plain drinking water. On day 9, 4 hours after the last administration of NTG (or vehicle) trigeminal hyperalgesia was assessed using the orofacial formalin test. All animals were then euthanized. Ileum and colon tissues, along with the trigeminal ganglia (TG) and cervical spinal cord, were used to assess the gene expression of tight junctions (TJs) and ECs components. In a separate cohort of rats, colon samples were collected for the immunohistochemical assessment of ZO-1 and occludin expression.

Results. Probiotic supplementation alleviated NTG-induced trigeminal hyperalgesia, significantly reducing nociceptive behaviour in the orofacial formalin test. NTG treatment compromised the integrity of the intestinal barrier, as evidenced by reduced expression of TJs in both colon and ileum. Probiotic supplementation restored these changes. NTG also induced region-specific changes in ECS components, including reduced expression of monoacylglycerol lipase (MAGL) in the ileum and the TG, increased cannabinoid receptor 2 expression in the ileum, and decreased diacylglycerol lipase (DAGL) expression in the colon. Probiotic supplementation restored MAGL gene expression in the ileum and TG and increased DAGL gene expression in the TG, suggesting a partial normalization of ECs signaling.

Conclusions. Chronic NTG-induced dysbiosis is associated with impaired intestinal barrier integrity and altered endocannabinoid signaling. Probiotic supplementation attenuates trigeminal hyperalgesia, restores ZO-1 expression, and normalizes MAGL levels in the intestine and TG, suggesting that modulation of 2-AG signaling contributes to gut-trigeminal communication.