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METABOLOMICS UNCOVERS DIETARY OMEGA-3 FATTY ACID-DERIVED METABOLITES IMPLICATED IN ANTI-NOCICEPTIVE RESPONSES AFTER EXPERIMENTAL SPINAL CORD INJURY☆

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Abstract—Chronic neuropathic pain is a frequent comorbidity following spinal cord injury (SCI) and often fails to respond to conventional pain management strategies. Preventive administration of docosahexaenoic acid (DHA) or the consumption of a diet rich in omega-3 polyunsaturated fatty acids (O3PUFAs) confers potent prophylaxis against SCI and improves functional recovery. The present study examines whether this novel dietary strategy provides significant antinociceptive benefits in rats experiencing SCI-induced pain. Rats were fed control chow or chow enriched with O3PUFAs for 8 weeks before being subjected to sham or cord contusion surgeries, continuing the same diets after surgery for another 8 more weeks. The paw sensitivity to noxious heat was quantified for at least 8 weeks post-SCI using the Hargreaves test. We found that SCI rats consuming the preventive O3PUFA-enriched diet exhibited a significant reduction in thermal hyperalgesia compared to those consuming the normal diet. Functional neurometabolomic profiling revealed a distinctive deregulation in the metabolism of endocannabinoids (eCB) and related N-acyl ethanolamines (NAEs) at 8 weeks post-SCI. We found that O3PUFAs consumption led to a robust accumulation of novel NAE precursors, including the glycerophospho-containing docosahexaenoyl ethanolamine (DHEA), docosapentaenoyl ethanolamine (DPEA), and eicosapentaenoyl ethanolamine (EPEA). The tissue levels of these metabolites were significantly correlated with the antihyperalgesic phenotype. In addition, rats consuming the O3PUFA-rich diet showed reduced sprouting of nociceptive fibers containing CGRP and dorsal horn neuron p38 mitogen-activated protein kinase (MAPK) expression, well-established biomarkers of pain. The spinal cord levels of inositols were positively correlated with thermal hyperalgesia, supporting their role as biomarkers of chronic neuropathic pain. Notably, the O3PUFA-rich dietary intervention reduced the levels of these metabolites. Collectively, these results demonstrate the prophylactic value of dietary O3PUFA against SCI-mediated chronic pain.

Key words: DHA, EPA, dietary fatty acids, endocannabinoid metabolome, spinal cord injury, chronic pain.

INTRODUCTION

Chronic neuropathic pain is one of the most important determinants in the perceived quality of life of spinal cord injury (SCI) patients (Anderson, 2004). Unfortunately, current therapeutics to treat this condition lack necessary efficacy and are limited in scope by unwanted side effects and poor tolerance. These shortcomings could be partly overcome with the use of preventive approaches that can provide resilience to damage prior to irreversible biochemical alterations occur in the perturbed cord. Trauma to the spinal cord triggers a robust secondary pathophysiological response, leading to cell death, inflammation, and dysfunction (Hulsebosch, 2002; Norenberg et al., 2004). Neuroinflammation is regarded as a hallmark mechanism underlying injury progression and pain processing (Christensen and Hulsebosch, 1997a; Hulsebosch et al., 2009), and thus represents an attractive target for therapeutic strategies (Kwon et al., 2011a,b).

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Abbreviations: 1-OG, 1-oleoyl glycerol; 2-AG, 2-arachidonoyl glycerol; 2-PG, 2-palmitoyl glycerol; Abh4, phospholipase A/B or α-β-hydrolase 4; AEA, arachidonoyl ethanolamine; BBB, Basso, Beattie and Bresnahan; CGRP, calcitonin gene-related peptide; DHA, docosahexaenoic acid; DHEA, docosahexaenoyl ethanolamine/synaptamide; DPEA, docosapentaenoyl ethanolamine; eCBs, endocannabinoids; EG, eicosenoyl glycerol; EPEA, eicosapentaenoyl ethanolamine; G3P, glycerol-3-phosphate; GAP43, growth-associated protein 43; GC/MS, gas chromatography/mass spectrometry; GDE1, glycerophosphodiesterase; GP-NAEs, glycerophospho-containing N-acyl ethanolamines; HWL, hindpaw withdrawal latency; LEA, linoleyl ethanolamine; LPA, lyso-phosphatidic acid; MAPK, mitogen-activated protein kinase; NAEs, N-acyl ethanolamines; NAPE, N-acyl phosphatidyl ethanolamine; O3PUFAs, omega-3 polyunsaturated fatty acids; PA, phosphatidic acid; PEA, palmitoyl ethanolamine; PLD, phospholipase D; PLS-DA, partial least square-discriminant analysis; SCI, spinal cord injury; TH, thermal hyperalgesia; UHPLC/MS/MS^2, ultrahigh performance liquid chromatography/tandem mass spectrometry.

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Dietary-essential omega-3 polyunsaturated fatty acids (O3PUFAs), such as docosahexaenoic acid (DHA), are integral components of neural membrane phospholipids and play crucial roles in anti-inflammatory responses (Calder, 2008). Longstanding studies have demonstrated that dietary PUFAs are mediating factors in pain processing, as evidenced by increased threshold for thermal pain and neuropathic pain in rats fed with high omega-3 to omega-6 PUFA ratios (Yehuda and Carasso, 1993). Recent studies have shown that O3PUFAs and their derivatives can exert strong antinociceptive effects against thermal and chemical stimulation in various animal models (Nakamoto et al., 2010; Xu et al., 2010; Tokuyama and Nakamoto, 2011). Given this evidence, it would seem reasonable to consider that dietary O3PUFAs may also play important roles in SCI-induced pain.

In a recent report, we showed that SCI causes a robust PUFA deregulation and leads to a marked DHA deficiency, which was associated with impaired recovery and dysfunction (Figueroa et al., 2013). Notably, administration of O3PUFAs maintained the cord PUFA homeostasis, conferred neuroprotection, prevented dysfunction and facilitated recovery after acute and chronic SCI, even when administered in a prophylactic manner (Figueroa et al., 2012, 2013). These findings led us to hypothesize that a preventive diet enriched in O3PUFAs modulates behavioral responses implicated in pathological nociception in rats. This idea is supported by studies showing that the diet type at the time of injury can affect pain behaviors associated with nerve lesions (Shir and Seltzer, 2001; Alloui et al., 2003; Hargraves and Hentall, 2005; Li et al., 2005; Estebe et al., 2006). Despite this evidence, diet remains a largely unexplored therapeutic avenue to ameliorate pain in SCI.

This study is an initial attempt to assess the effects of dietary O3PUFAs on thermal pain stimuli in SCI rats. Because N-acylated ethanolamines (NAE) and related endocannabinoids (eCBs) are bioactive lipids implicated in pain processing (Beltramo et al., 2006; Petrosino et al., 2007; Hama and Sagen, 2011), we focused on identifying the involvement of dietary O3PUFAs in their local modulation following SCI. Here, the eCB metabolome has been expanded to include the ethanolamines, glycerides, and metabolic precursors, intermediates, and derivatives. These metabolites have been implicated in regulating anti-inflammatory responses and can exert cannabimimetic actions as endogenous agonists of cannabinoid receptors (Devane et al., 1992; Hanus et al., 1993; Priller et al., 1995; Brown et al., 2010), but whether dietary PUFAs impact the levels of these bioactive lipids in SCI has not been comprehensively evaluated. To address this issue, we employed both LC/MS and GC/MS-based metabolomics on cord samples collected from sham and contusion SCI-operated Sprague–Dawley rats that received either control or O3PUFA-enriched diets. Deciphering the neurometabolomic profile that distinguishes pain-like behaviors may have important clinical implications for pain management and allow for improved prognosis in SCI.

EXPERIMENTAL PROCEDURES

Animals

All animal studies were performed in compliance with the Loma Linda University School of Medicine regulations and institutional guidelines consistent with the NIH Guide for the Care and Use of Laboratory Animals. Female Sprague–Dawley rats were received from Charles River Laboratories (Portage, MI) and housed in individual cages on alternating 12-h light/ dark cycles. It is worth noting that in this study we used two independent cohorts of animals. Although both cohorts received the same dietary and surgical interventions, and behavioral testing, there were differences in the time allowed for survival (cohort 1: at least four animals per diet group, allowed to survive until 12 weeks post-injury; cohort 2: at least 13 animals per diet group, allowed to survive until 8 weeks post-injury). Animals in cohort 2 were also used to determine the effect of dietary O3PUFAs in sensorimotor and autonomic dysfunction after SCI (Figueroa et al., 2013).

Diet composition

Custom AIN-93-based diets were prepared with modifications to the fat composition as described previously (Figueroa et al., 2013). Briefly, dietary fats were approximately 6% of the pellets dry weight and were supplied as either soybean oil (control chow) or menhaden fish oil (O3PUFA-enriched chow: DHA = 12.82-gm and EPA = 6.91-gm per 100 gm of diet). Diets were matched for cholesterol content.

Surgical and post-operative procedures

Eight weeks after the dietary pretreatment, animals were deeply anesthetized with a mixture of ketamine/xylazine (80 and 10 mg/kg, respectively). The spinal cord injuries were generated using the well-characterized New York University (NYU) Impactor (Gruner, 1992). Notably, trauma caused using this device induces below-level pain that is well developed and longstanding, suggesting that the model is suitable for chronic pain research (Jung et al., 2008). To produce the contusion, the skin and the muscles overlying the spinal column were cut. A laminectomy was performed at the T9–T10 level and the T8 and T12 spinal processes were clamped to the Impactor, and the exposed dorsal surface of the cord was subjected to weight drop impact using a 10-g rod released from a height of 12.5-mm. Sham animals received only a laminectomy. The animals body temperature was maintained at 37 °C during the procedure. After operation, muscle layers were sutured and skin layers closed. The bladders of injured rats were expressed using the Crede's maneuver three times a day until voiding reflexes were restored. Cefazolin (Bristol Myers Squibb, New York, NY; 25 mg/kg, s.q.) and Buprenex® (buprenorphine; Reckett and Colman Pharmaceuticals, Inc. Richmond, VA; 0.05 mg/kg, s.c.) were given to all rats for 5 and 3 consecutive days, respectively. Animals were allowed to survive for