

Oral supplementation with the omega-3 fatty acids docosahexaenoic and eicosapentaenoic attenuates mechanical allodynia in a mouse model of peripheral nerve injury

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Abstract

Objective: The omega-3 fatty acids docosahexaenoic (DHA) and eicosapentaenoic (EPA) are precursors to a family of analgesic and neuroprotective small pro-resolution lipid mediators (PRLMs) that include the resolvins and neuroprotectins. We hypothesized that perioperative supplementation with DHA and EPA can prevent post-surgical pain by increasing endogenous levels of PRLMs.

Methods: We treated mice undergoing spared tibial nerve injury with perioperative oral DHA and EPA with or without aspirin to determine whether DHA, EPA, and their PRLM metabolites can reduce mechanical allodynia in a mouse model of peripheral nerve injury.

Results: We found that mice treated with both DHA/EPA or DHA/EPA with aspirin had significantly reduced mechanical allodynia in the ipsilateral paw compared to injured control animals. There was no significant difference in allodynia reduction between the treatment groups.

Conclusion: Perioperative DHA/EPA supplementation reduces mechanical allodynia in a mouse model of peripheral nerve injury and may be a safe, cost-effective adjunct to prevent or treat post-injury neuropathic pain in humans.

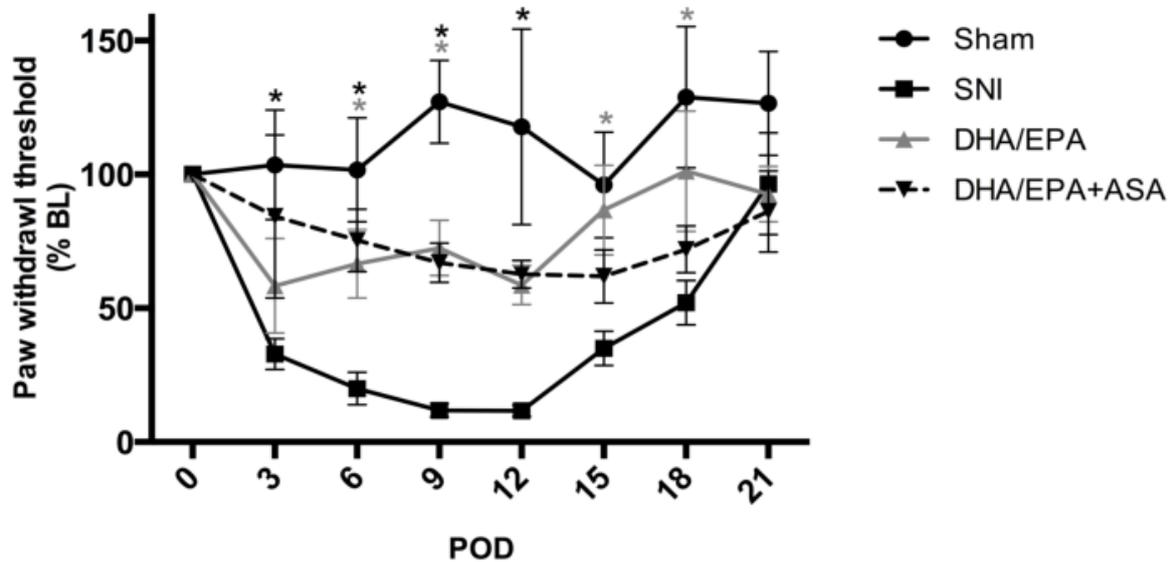
Introduction

Surgical procedures, including mastectomy, amputation, and thoracotomy, are followed by severe and disabling chronic neuropathic pain in 5–10% of cases [1]. Despite our success at managing acute post-surgical pain, current therapeutic options for chronic pain are limited. Since this type of chronic neuropathic pain is believed to partially result from neuroinflammation following nerve injury and the subsequent peripheral and central sensitization that occurs as a consequence of this inflammation [1] [2] [3], novel therapeutic strategies aimed at interrupting this inflammation and switching to a resolving state seem especially promising. Growing evidence suggests that the novel pro-resolving lipid mediator (PRLM) metabolites of the omega-3 fatty acids docosahexaenoic (DHA) and eicosapentaenoic acids (EPA) have significant anti-inflammatory, pro-resolution, and analgesic effects [4] [5]. These PRLMs, which include resolvins, neuroprotectins, and maresins, are generated via several metabolic pathways involving lipoxygenase (LOX) or cyclooxygenase (COX) activity at sites of tissue injury [6] [7]. Of note, two R-series resolvins are generated from DHA and EPA by the aspirin acetylated COX2 enzyme [6]. Resolvins and neuroprotectins have been extensively studied in multiple rodent models of pain and found to prevent as well as treat established inflammatory, post-surgical, and neuropathic pain [8]. Unfortunately, PRLMs are relatively unstable and costly to produce. Therefore, we hypothesized that by providing the upstream PRLM precursors, DHA and EPA, to enhance PRLM production at the site of injury, the development of mechanical allodynia after peripheral nerve injury in mice may be attenuated.

Objective

Small pro-resolution lipid mediators (PRLMs) are powerful anti-inflammatory and analgesic molecules derived from the omega-3 fatty acids docosahexaenoic (DHA) and eicosapentaenoic (EPA). Since these PRLMs are unstable and difficult to produce, we decided

to treat mice undergoing peripheral nerve injury with oral DHA and EPA to determine if large amounts of these PRLM precursors given orally would attenuate mechanical allodynia development.



a

Figure Legend

Figure 1. Perioperative supplementation with DHA and EPA attenuates mechanical allodynia following spared nerve injury.

Paw withdrawal thresholds are presented as mean percent of baseline \pm SEM, from baseline (POD0) through post-operative day (POD) 21. Lower thresholds represent mechanical allodynia. Mice treated with DHA and EPA (DHA/EPA, DHA/EPA + ASA) developed significantly less mechanical allodynia (50–60%) at the majority of time points compared to the spared nerve injury control group (SNI) using repeated-measures two-way ANOVA followed by Tukey's post-hoc test. *Represents significant difference of treatment group from SNI ($p < 0.05$).

Animals

8 to 10 week-old male C57BL/6 mice ordered from Charles River Laboratories (Wilmington, MA) were used. The mice were housed in cages (five in each) and had access to chow and water *ad libitum*. The chow was either Picolab[®] Rodent Diet 20 (5053) or Rodent Laboratory Diet (5001) from LabDiet[®] (St. Louis, MO), containing both of which contain omega-3 to omega-6 PUFA ratio of about 0.15.

Drug Administration

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were purchased from Cayman Chemical as solutions in ethanol. For these experiments, after evaporating the ethanol solvent under a gentle nitrogen stream, DHA and EPA were delivered in a soybean oil control vehicle (Crisco Pure Vegetable Oil) containing the anti-oxidant vitamin E. Soybean oil was chosen as the control vehicle for its low omega-3 to omega-6 PUFA ratio of 0.14, which is similar to the mice's background diet. Acetylsalicylic acid (ASA) was purchased from Sigma. All treatments were delivered daily to the mice via oral gavage, on the day before surgery through post-operative day 12. Mice in the appropriate treatment groups received approximately 200 mg/kg of DHA/EPA and 30 mg/kg of ASA per treatment. Dosing estimates were based on, the average mass of an adult C57BL/6 mouse of 26 g. Control groups received soybean oil over the same time span. DHA and EPA were delivered together in the soybean oil carrier. ASA was delivered in water.

STNI Surgery

STNI surgery was performed under 2–3% isoflurane anesthesia, as described by Shields [16]. The left hind limb was immobilized in a lateral position. After skin incision at the

mid-thigh level and dissection through the underlying muscle, the sciatic nerve trifurcation was exposed. The common peroneal and sural nerve branches were tightly ligated with 6-0 silk sutures and then severed. Throughout the procedure, the tibial nerve was preserved by carefully avoiding any stretch or nerve contact. For sham surgeries, the sciatic nerve trifurcation was exposed without imposing any nerve injury.

Behavioral Assays

Mice were habituated to the testing environment for 2 days prior to baseline testing and for at least 30 min on each subsequent test day. The mice were placed in plastic boxes on an elevated wire-mesh apparatus. Mechanical allodynia was assessed by stimulating the left hind paw with Von Frey filaments of logarithmically increasing stiffness (0.04–2.00 g, Stoelting Co, Wood Dale, IL), applied perpendicularly to the plantar surface. Specifically, the hindpaw was stimulated in the distribution of the tibial nerve, in the center of the plantar surface. The 50% paw withdrawal thresholds were determined using the up-down method of Dixon [17]. Testing was performed by a blinded researcher at baseline, post-operative day (POD) 3 and every following third day, finishing on POD 21.

Statistical analysis

Statistical analysis of mouse behavioral data was performed in GraphPad. Paw withdrawal thresholds were normalized to baseline and are presented as mean percentage of baseline with standard error of the mean. Missing data was imputed with the mean of the shared treatment group's paw withdrawal threshold for the time point. The paw withdrawal thresholds of the treatment groups were compared using ANOVA and ad hoc Tukey tests corrected for multiple comparisons. **Results & Discussion**

To test the effects of DHA and EPA supplementation with and without aspirin on mechanical allodynia in mice with surgically induced peripheral nerve injury, we created four groups of 10 mice each. One group underwent sham surgery and received daily control soybean oil. The remaining groups all underwent STNI surgery and received daily control soybean oil, DHA and EPA, or DHA, EPA, and aspirin. We assessed the influence of omega-3 fatty acid supplementation on post-operative allodynia by comparing the groups' mean paw withdrawal thresholds across all time points (Fig. 1). As anticipated, the SNI control mice developed significant mechanical allodynia by POD3 ($p < 0.0001$) that peaked on PODs 9–12 and resolved by POD21. We found that treatment with DHA and EPA significantly attenuated the development of mechanical allodynia compared to SNI controls. The DHA/EPA group had improved allodynia compared to SNI on PODs 6, 9, and 15 ($>50\%$, $p < 0.05$) and trended towards improved outcome on POD12 (35.2%, $p = 0.055$). Similarly, the treatment group with aspirin had substantially improved allodynia compared to SNI on PODs 3–12 ($>50\%$, $p < 0.05$). Aspirin did not appreciably augment the therapeutic effect of omega-3 PUFA supplementation, as there were no significant differences between the two treatment groups.

Mechanistic understanding of the transition from acute to chronic neuropathic pain after nerve injury has advanced significantly over the past decade, but advances in preventive therapeutics continue to be slow. Recently, DHA- and EPA-derived pro-resolution lipid mediators (PRLMs) have been shown to have powerful analgesic and anti-inflammatory effects [9]. In addition, the PRLM NPD1 was found to provide preventive analgesia in animal models of post-nerve injury pain has provided one of the most intriguing leads toward novel preventive therapeutics [7] [10]. The PRLMs themselves are short-lived and difficult to produce in quantity, but supplementation with their omega-3 fatty acid precursors offers a possible way to increase the concentration of these small lipid mediators at the target injury site [11]. In addition, aspirin acetylation of cyclooxygenase has been shown to increase the production of a group of aspirin-derived PRLMs that may further increase the analgesic efficacy of DHA/EPA supplementation [6] [12]. The potent analgesic effects of PRLMs and the difficulty inherent in producing and treating with PRLMs themselves prompted us to study whether supplementation of DHA/EPA in mice undergoing peripheral nerve injury (including the nerve transection that occurs in amputation) would reduce post-nerve injury allodynia. Therefore, we provided oral supplementation of DHA and EPA to mice before and after spared tibial nerve injury (STNI) and found that these injured mice had significantly

reduced post-injury mechanical allodynia and a trend towards higher plasma neuroprotectin levels. This reduction in allodynia continued for 6 days after oral DHA/EPA supplementation was discontinued. By day 21, all four experimental groups returned to baseline.

Though we did not directly measure plasma PRLM levels in treated mice, previous studies have demonstrated that supplementation with DHA and EPA translates to increased endogenous resolvin levels in healthy human volunteers [12] [13]. Also, there is growing evidence that increasing the ratio of omega-3 to omega-6 fatty acids likely augments the beneficial effects of omega-3 PUFA supplementation on multiple disease states [14]. Recent work by Ramsden et al. supports the idea that dietary supplementation with omega-3 fatty acids increases the blood concentration of these small lipid mediators while reducing pain symptoms [15]. This clinical trial concluded that migraine patients receiving a high omega-3 and low omega-6 fatty acid diet had significantly higher blood levels of the immediate precursors to resolvin and neuroprotectin biosynthesis and also increased resolvin D2 concentration. They also found that this dietary intervention reduced the incidence of migraine headache. Reducing omega-6 fatty acids in the mouse diet in addition to DHA/EPA supplementation may produce more dramatic improvements in mechanical allodynia than with omega-3 supplementation alone.

Conclusions

We found that oral DHA and EPA supplementation reduced mechanical allodynia after peripheral nerve injury in mice. This finding suggests that perioperative DHA/EPA supplementation may provide a safe, inexpensive, and effective way to increase pro-resolving lipid mediator levels and prevent chronic pain in humans undergoing surgery such as thoracotomy and amputation that involves peripheral nerve injury. Given the low-risk of side effects and current high prevalence of use, omega-3 PUFA supplementation could easily be applied perioperatively.

Limitations

Though allodynia reduction in the two DHA/EPA treatment groups was dramatic, it is difficult to conclude from this data whether supplementation acts as a therapeutic or as a preventive therapeutic due to the inherent improvement of mechanical allodynia over time in this particular peripheral nerve injury model. There are several models of peripheral nerve injury that produce more dramatic and long-lasting allodynia that could be used in future studies to verify that DHA/EPA supplementation is preventive and not just therapeutic. It is also unclear why aspirin treatment did not further reduce mechanical allodynia in this mouse model. A possible explanation is that DHA and EPA might themselves be analgesic and anti-inflammatory beyond their role as precursors to PRLMs. This direct analgesic effect could act to dilute the consequences of the small increase in PRLM production provided by aspirin. There is evidence that free fatty acids like DHA and EPA have specific receptors, such as free-fatty acid receptor-4, that transduce direct and significant anti-inflammatory changes independent of the activities of the PRLMs [14].

Additional Information

Methods

Animals

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Supplementary Material

Please see <https://sciencematters.io/articles/201602000029>.

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Ethics Statement

All animal experiments were approved by the Institutional Animal Care and Use Committee at Duke University and were conducted in accordance with the U.S. Government Principles for Utilization and Care of Vertebrate Animals for Testing, Research, and Training.

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