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

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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 (SNI) with perioperative oral DHA and EPA with or without aspirin to determine whether DHA, EPA and their PRLM metabolites 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, economical adjunct to prevent or treat post-injury neuropathic pain in humans.

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 precursor given orally would attenuate mechanical allodynia development.

Introduction
Surgical procedures, including mastectomy, amputation, and thoracotomy, are followed by severe and disabling chronic neuropathic pain in 5-10% of cases. Despite our success at managing acute post-surgical pain, current therapeutic options for the chronic pain that follows 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, 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. 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. Of note, there are two R-series resolvins that are generated from DHA and EPA by the aspirin acetylated COX2 enzyme. 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. 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.
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Perioperative supplementation with DHA and EPA attenuates mechanical allodynia following spared-nerve injury. Paw withdrawal thresholds are presented as mean percent of baseline ± 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).
Results & Discussion

Results

To test the effect of DHA and EPA supplementation with and without aspirin on mechanical alldynia in mice with surgically induced peripheral nerve injury, we created 4 groups of 10 mice each. One group underwent sham surgery and received daily control soybean oil. The remaining groups all underwent SNI 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 alldynia by comparing the groups’ mean paw withdrawal thresholds across all time points (Figure 1). As anticipated, the SNI control mice developed significant mechanical alldynia 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 alldynia compared to SNI controls. The DHA/EPA group had improved alldynia compared to SNI on PODs 6, 9, and 12 (>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 alldynia compared to SNI on PODs 3 through 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.

Discussion

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.[10] 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.[9] 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.[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 alldynia. Therefore, we provided oral supplementation of DHA and EPA to mice before and after spared-tibial nerve injury and found that these injured mice had significantly reduced post-injury mechanical alldynia and a trend toward higher plasma neuroprotectin levels. This reduction in alldynia 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.[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 alldynia than with omega-3 supplementation alone.

Conclusions

We found that oral DHA and EPA supplementation reduced mechanical alldynia 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 that involves peripheral nerve injury such as thoracotomy and amputation. Given the low-risk of side effects and current high prevalence of use, omega-3 PUFAs supplementation could easily be applied perioperatively.

Limitations

Though alldynia 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 alldynia over time in this particular peripheral nerve injury model. There are several models of peripheral nerve injury that produce more dramatic and long-lasting alldynia 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 alldynia 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
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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 (Moniri 2016).
Additional Information

Methods and Supplementary Material
Please see https://medicalmatters.io/articles/201602000029.

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Ethics Statement
All animal experiments were approved by the Institutional Animal Care & 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

Citations


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