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Fish oil improves motor function, limits blood–brain barrier disruption, and reduces *Mmp9* gene expression in a rat model of juvenile traumatic brain injury

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ABSTRACT

The effects of an oral fish oil treatment regimen on sensorimotor, blood–brain barrier, and biochemical outcomes of traumatic brain injury (TBI) were investigated in a juvenile rat model. Seventeen-day old Long-Evans rats were given a 15 mL/kg fish oil (2.01 g/kg EPA, 1.34 g/kg DHA) or soybean oil dose via oral gavage 30 min prior to being subjected to a controlled cortical impact injury or sham surgery, followed by daily doses for seven days. Fish oil treatment resulted in less severe hindlimb deficits after TBI as assessed with the beam walk test, decreased cerebral IgG infiltration, and decreased TBI-induced expression of the *Mmp9* gene one day after injury. These results indicate that fish oil improved functional outcome after TBI resulting, at least in part from decreased disruption of the blood–brain barrier through a mechanism that includes attenuation of TBI-induced expression of *Mmp9*.

1. Introduction

Traumatic brain injury (TBI) is a major cause of death and acquired disability in young children [1]. Despite having a high degree of neuroplasticity, young children tend to have poorer outcomes after TBI than adults [2]. Children can also exhibit differential responses to pharmacological interventions including altered bioavailability, metabolism, and drug response [3,4]. It is thus critical that both the effect of TBI and potential therapeutics be investigated in an age-appropriate model [5].

The n-3 long-chain polyunsaturated fatty acids (LC-PUFA) eicosapentaenoic acid (EPA, 20:6n-3) and docosapentaenoic acid (DHA, 22:6n-3), constituents of fish oil, are biologically active with many neuroprotective properties. When consumed in the diet or via supplementation, these n-3 fatty acids are incorporated into the phospholipids that form cell membranes where they can influence the physicochemical and membrane-signaling properties of the cell [6,7]. DHA, EPA and their metabolites also have anti-excitotoxic [8], antioxidant [9], anti-apoptotic [10,11], and anti-inflammatory properties [12]. During development, N-3 fatty acids, particularly DHA, accumulate in the brain during late gestation and early neonatal life in humans and rats, a time at which children have a high risk for sustaining traumatic brain injuries [13–15]. Additionally, EPA and DHA can be metabolized into several families of molecules including NPD1, docosanoids, resolvins, which have been shown be neuroprotective through their anti-inflammatory and inflammation-resolving activities [16–18]. LC-PUFA can also directly or indirectly modulate gene expression through activation or suppression of cell signaling pathways and transcription factors (e.g., PI3K/Akt, NF-κB, PPAR and RXR) [19–21].

DHA and EPA, the primary active constituents in fish oil, readily cross the blood–brain barrier [22]. Studies of various neural injury models including TBI and spinal cord injuries in adult animals indicate that fish oil, or DHA or EPA alone produce beneficial effects [23–25]. Furthermore, in a case report, high dose fish oil supplementation (19.2 g/day) was associated with substantial clinical improvement in a young patient with severe, potentially lethal, head trauma [26]. However, the effects of LC-PUFA or fish oil treatment have not been investigated in juvenile brain injury. Accordingly, this study investigated the use of oral fish oil treatment in a juvenile rat model on sensorimotor and biochemical outcomes of TBI, including blood–brain barrier disruption and levels of Ccl_2, *Gfap*, and *Mmp9* mRNAs, representative key mediators involved in immune cell recruitment, astrocytosis, and blood–brain barrier

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disruption after TBI. We will show that fish oil resulted in improved functional outcome, at least in part, by limiting disruption of the blood–brain barrier through a mechanism that includes attenuation of TBI-induced expression of *Mmp9*.

2. Materials and methods

All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Kansas Medical Center Institutional Animal Care and Use Committee.

2.1. Animals, husbandry, and treatment

Long-Evans rats were housed in a temperature- and humidity-controlled facility with a 14-10 hour light-dark cycle (on at 06:00 h) with ad libitum access to water and chow (Teklad Global diet 2016, Harlan Laboratories, Inc., Indianapolis, IN). The chow contained 4% fat by weight and was formulated with soybean oil resulting in a fatty acid composition of 0.5% 16:0, 0.1% 18:0, 0.7% 18:1n-9, 2% 18:2n-6, 0.1% 18:3n-3. Breeding stock (females 75–85 days; male proven breeders; Harlan Laboratories, Inc. Indianapolis, IN) were obtained a minimum of 5 days prior to the beginning of the experiment and were handled regularly. Litters were culled to eight pups with preference for males on postnatal day (PND) one. Male pups ($n=4$–12/group, depending on endpoint, and each from a different litter) received either a controlled cortical impact (CCI) injury or sham surgery on PND 17 and were returned to the dam until weaning. Pups were weaned on PND 20 and housed in groups of two to four, TBI and sham-injured together, for the remainder of the study.

Rats were treated with either 15 mL/kg of fish oil (2.01 g/kg EPA, 1.34 g/kg DHA; Nature Made 1200 mg fish oil capsules, Mission Hills, CA) or unhydrogenated soybean oil via oral gavage 30 min prior to the initial TBI or Sham surgery, and then daily for seven days. On days behavioral testing occurred (see below), oil was administered after testing to avoid any confounding effect the gavage procedure might have on behavior. On the day of euthanasia, rats were decapitated without anesthesia approximately six hours after oil administration. Soybean oil was used as the comparator in this study to control for the caloric content of the fish oil, and, because it is the oil used in the formulation of the Teklad Global #2016, it does not introduce any fatty acids not already consumed by rats in both treatment groups. The fatty acid composition of the oils is shown in Table 1.

Rats used for assessment of sensorimotor function were tested 1, 4, and 7 days after surgery and then euthanized on day 7 by transcardial perfusion under pentobarbital anesthesia, followed by removal of the brain for IgG immunohistochemistry. Rats used for determination of mRNA levels were euthanized on day 1 (28 h after surgery) or day 4 after surgery by decapitation. Brains from these rats were rapidly removed and then dissected on ice. The frontal cortex, a cortical region remote from the contusion site, which was not needed for mRNA analyses and has a fatty acid composition representative of whole brain [27], was frozen on dry ice for later fatty acid analysis. The injured motor cortex was preserved in RNAlater (Life Technologies/Ambion, Gaithersburg, MD) for mRNA analysis.

2.2. Controlled cortical impact

CCI was performed as previously described [28,29]. Briefly, rats were stabilized in a Cunningham stereotaxic frame (Stoelting, Wood Dale, IN) after being anesthetized with isoflurane (induction, 3.0%; maintenance, 2.0%). A 4×4 mm craniotomy was performed lateral (right side) to the mid-sagittal suture, centered at: AP=0, ML=2.5 from bregma. The impactor device, previously described in detail [30] was equipped with a 3.0 mm-diameter tip. The impactor tip was centered within the craniotomy and lowered until the tip just contacted the dura over motor (M1, M2) and sensory (S1FL, S1HL) cortical areas [31,32]. The parameters of the impact were as follows: 3.0 mm depth, 1.5 m/sec strike velocity, 300 msec contact time. The scalp incision was then closed with a 6-0 silk suture and the animal was allowed to recover from anesthesia.

Sham procedures involving the use of a trephine or drill to produce a craniotomy have been shown to cause brain injury distinct from that caused by the impact [33,34]. Though cortical damage caused by TBI significantly outweighs damage produced by the craniotomy [35], the sham surgery consisted of an incision in the scalp with no craniotomy or impact from the CCI device to avoid potential experimental confounds that might be caused by any additional intervention.

All rats received 0.05 mg/kg of buprenorphine approximately 30 min after surgery and again 24 h after surgery, after day 1 behavioral testing was completed. The administration of buprenorphine analgesia after CCI surgery was required by the Institutional Animal Care and Use Committee. Buprenorphine is a μ-opioid receptor partial agonist and is thus analgesic, but not anti-inflammatory [36]. Opioid analgesics are clinically contraindicated for use in TBI because they can increase intracranial pressure thus exacerbating the effects of injury; however, the craniotomy required for the CCI likely minimizes this effect. Furthermore, the administration of buprenorphine to all treatments groups ensures that any effects it may produce are controlled for.

Table 1
Fish and soybean oil fatty acid composition.

Percent of total fatty acids (wt%)		
Fatty acid	**Fish oil**	**Soybean oil**
14:0	7.73	ND
16:0	15.95	11.12
16:1	13.63	0.10
18:0	5.18	5.05
18:1n-9c	10.26	20.99
18:2n-6c	3.63	50.10
18:3n-6	0.45	ND
18:3n-3	0.77	9.74
20:1n-9	1.04	0.13
20:2n-6	0.37	0.32
20:3n-6	0.23	ND
20:4n-6	1.70	0.02
22:1	0.22	0.04
20:5n-3	11.57	ND
22:2n-6	0.13	0.27
22:5n-6	0.36	ND
22:6n-3	16.39	ND

ND: not detected.

2.3. Beam walk test

Rats were tested for their ability to traverse a 75 cm-long wooden dowel (15 mm diameter) elevated 30 cm and ending in a dark goal box as previously described in detail [29]. Video-taped sessions were scored for ipsilateral and contralateral foot slips, time required to reach the goal box, and total number of steps taken by the right (uninjured) hind foot. Data are reported as the percent of contralateral foot slips relative to the total number of steps needed to traverse the beam.