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Increased brain docosahexaenoic acid has no effect on the resolution of neuroinflammation following intracerebroventricular lipopolysaccharide injection

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ABSTRACT

Resolution of inflammation in the periphery was once thought to be a passive process, but new research now suggests it is an active process mediated by specialized pro-resolving lipid mediators (SPM) derived from omega-3 polyunsaturated fatty acids (n-3 PUFA). However, this has yet to be illustrated in neuroinflammation. The purpose of this study was to measure resolution of neuroinflammation and to test whether increasing brain docosahexaenoic acid (DHA) affects the resolution of neuroinflammation.

C57Bl/6 mice, fat-1 mice and their wildtype littermates, fed either fish oil or safflower oil, received lipopolysaccharide (LPS) in the left lateral ventricle. Animals were then euthanized at various time points for immunohistochemistry, gene expression, and lipidomic analyses.

Peak microglial activation was observed at 5 days post-surgery and the resolution index was 10 days. Of the approximately 350 genes significantly changed over the 28 days post LPS injection, 130 were uniquely changed at 3 days post injection. No changes were observed in the bioactive mediator pools. However, a few lysophospholipid species were decreased at 24hr post surgery. When brain DHA is increased, microglial cell density did not resolve faster and did not alter gene expression.

In conclusion, resolution of neuroinflammation appears to be independent of SPM. Increasing brain DHA had no effect in this model.

1. Introduction

Neuroinflammation is a characteristic of many neurological and psychiatric disorders. *In vivo* and postmortem studies have both reported increased neuroinflammation in Alzheimer's disease, Parkinson's disease, and schizophrenia (Glass et al., 2010; Najjar et al., 2013; Trepanier et al., 2016b). Growing evidence suggests a potential causal effect of neuroinflammation in the progression of the pathogenesis of these and other neurological and psychiatric disorders (Glass et al., 2010; Heneka et al., 2015).

The brain is an immunological different tissue compared to the periphery, containing its own resident immune cell in the microglia (Li and Barres, 2018). Microglia survey the environment, and communicate with other glia and neurons. Following insults or tissue damage, microglia are activated and release pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β (Cherry et al., 2014; Chhor et al., 2013). These signals activate astrocytes to release more pro-inflammatory cytokines including IL-1β (Liddelow et al., 2017). When chronic inflammation persists, neuronal death ensues. Microglia, however, can be also be activated by IL-4 and IL-13 and release anti-inflammatory cytokine IL-10 and growth factors such as insulin growth factor β and transforming growth factor β (Cherry et al., 2014; Chhor et al., 2013).

Classically, it was thought that inflammation dissipated passively. It is becoming clear, however, that the resolution of inflammation is an active process (Schwab et al., 2007; Serhan, 2014). Resolution of inflammation in the periphery is driven by specialized pro-resolving lipid mediators (SPM) derived from the enzymatic oxygenation of

Abbreviations: CCL, chemokine (c-c motif) ligand; CD, cluster of differentiation; COX, cyclooxygenase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GFAP, glial fibrillary acidic protein; i.c.v., intracerebroventricular; Iba, ionized calcium-binding adapter molecule; IL, interleukin; LN2, lipocalin 2; LPS, lipopolysaccharide; PUFA, polyunsaturated fatty acids; SPM, specialized pro-resolving lipid mediators; SAA3, serum amyloid A3; TNF, tumor necrosis factor; TSPO, translocator protein 18 kDa

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polyunsaturated fatty acids (PUFA) (Serhan, 2014; Serhan et al., 2015). SPM are considered both anti-inflammatory and pro-resolving. In the periphery, SPM actively return the inflamed tissue to homeostasis by blocking neutrophil entry and activating the recruitment of macrophages to repair the tissue and clear debris. While omega n-6 PUFA are typically considered pro-inflammatory due to being precursors of prostaglandins and leukotrienes, they can also be converted to the SPM lipoxin. Through the enzymatic activity of lipoxygenases, n-3 PUFA also produce SPM including protectins, resolvins, and maresins. Due to the differences in the cellular machinery controlling inflammation in the periphery and neuroinflammation, it is unknown, however, whether resolution of neuroinflammation also utilizes SPM.

In the brain, n-3 PUFA make up approximately 10% of all fatty acids (Brenna and Diau, 2007; Lin et al., 2015). Docosahexaenoic acid (DHA) is the most abundant n-3 PUFA in the brain and is involved in regulating neuronal and glial structure, while also serving as precursor to signaling molecules. Due to its abundance and multiple functions in the brain, it is not surprising that a link between n-3 PUFA and both neurological and psychiatric disease has been proposed and investigated (Amminger et al., 2010; Appleton et al., 2015; Dyall, 2015; Freeman et al., 2006; Song et al., 2016). Observational studies have suggested a protective role of n-3 PUFA in multiple brain disorders, such as Alzheimer's disease and depression (Beydoun et al., 2013; Hashimoto et al., 2014; Lin et al., 2012; Zhang et al., 2016). The results from clinical trials, however, are conflicting (Appleton et al., 2015; Burckhardt et al., 2016; Joy et al., 2006; Quinn et al., 2010) with only a few studies pointing to a protective effect (Amminger et al., 2010; Freund-Levi et al., 2006; Peet and Horrobin, 2002).

There have been several mechanisms proposed for the protective effects of n-3 PUFA in neurological and psychiatric disorders. These include anti-apoptotic, neurotrophic, and anti-oxidative mechanisms (Bazinet and Laye, 2014). Another potential mechanism of n-3 PUFA involves their anti-neuroinflammatory actions (Bazinet and Laye, 2014; Laye et al., 2018). N-3 PUFA have anti-inflammatory properties in a multitude of *in vivo* disease models including stroke, spinal cord injury, Alzheimer's disease and Parkinson's disease (Trepanier et al., 2016c). Increased brain DHA, either through dietary intervention or in the fat-1 mouse, has decreased pro-inflammatory gene expression 24 h following intracerebroventricular (i.c.v.) injection of lipopolysaccharide (LPS). Moreover, i.c.v. injection of 17S-hydroperoxyDHA, a precursor of protectin D1, had a more potent effect than DHA itself, suggesting that some or all of the anti-neuroinflammatory effects of DHA may be mediated by its metabolism to protectin D1 (Orr et al., 2013). This is consistent with the anti-neuroinflammatory effects of protectin D1, aspirin-triggered resolvin D1, resolvin E1, and resolvin D2 in stroke (Bazan et al., 2012; Marcheselli et al., 2003), Parkinson's disease (Tian et al., 2015), traumatic brain injury (Harrison et al., 2015) and neuropathic pain models (Xu et al., 2013a, 2013b). Similarly, *in vitro* experiments have demonstrated that SPM to have pro-resolving effects including the inhibition of pro-inflammatory mediator production, peroxisome proliferator activated receptor gamma activation, and the modulation of microglia morphology (Li et al., 2014; Rey et al., 2016).

Despite the fact that animal studies have generally pointed to anti-neuroinflammatory properties of n-3 PUFA, not much is known regarding their effects on resolution, as most studies have evaluated only a few pro-inflammatory markers at one time point (Trepanier et al., 2016c). It is therefore possible that the effects of n-3 PUFA may have been missed if the wrong marker or time point was chosen.

As there are many immunological differences between the brain and the rest of the periphery, the main goal of this study identify the key players in the resolution of neuroinflammation following i.c.v. LPS over 28 days utilizing immunohistochemistry, microarray and lipidomic approaches (Experiment 1). Once this is determined, the second goal of this study was to evaluate whether resolution of neuroinflammation is influenced by increasing brain DHA (Experiment 2).

Table 1
Percent of total fatty acids of the 3 experimental diets.

	Fish oil	Safflower oil	Chow
C12:0	0.01	0.01	0.00
C14:0	1.97	0.29	0.10
C14:1	0.06	0.00	4.37
C16:0	10.92	8.56	8.88
C16:1	2.16	0.14	0.13
C18:0	5.01	6.21	2.68
C18:1 n-9	14.64	13.35	18.93
C18:1 n-7	0.64	1.55	0.68
C18:2 n-6	58.75	67.25	56.44
C18:3 n-6	0.10	0.01	0.17
C18:3 n-3	0.43	0.20	6.46
C20:0	0.39	0.44	0.24
C20:1 n-9	0.55	0.33	0.33
C20:2 n-6	0.20	0.13	0.16
C20:3 n-6	0.03	0.00	0.01
C20:4 n-6	0.11	0.00	0.00
C20:3 n-3	0.03	0.00	0.00
C20:5 n-3	1.51	0.12	0.00
C22:0	0.36	0.42	0.22
C22:1 n-9	0.28	0.30	0.09
C22:5 n-6	0.05	0.00	0.00
C22:5 n-3	0.22	0.01	0.00
C22:6 n-3[a]	1.37	0.01	N.D
C24:1 n-9	0.21	0.17	0.00

[a] Composition was confirmed by gas chromatography with mass spectrometry.

2. Materials and methods

The present experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and were approved by the Animal Care Committee of the Faculty of Medicine of the University of Toronto. Animals were housed 1–4 per cage in our animal facility where temperature (21 °C) and light (14/10 light/dark cycle) were controlled. Food and water were available *ad libitum*.

2.1. Diets

Animals were fed one of 3 diets, 1) rodent chow (Teklad Global Diets, Envigo, Madison, WI), 2) modified AIN-93-G diets with 10% safflower oil (D04092701, Research diets, New Brunswick, NJ), or 3) modified AIN-93-G diets with 8% safflower and 2% menhaden oil (D04092702, Research Diets, New Brunswick, NJ).

Diet fatty acid composition was confirmed in triplicate by gas chromatography flame ionization detection and is presented in Table 1. The rodent chow contained 18.9% oleic acid, 56.4% linoleic acid and 6.5% α-linolenic acid as a % of total fatty acids. Eicosapentaenoic acid (EPA) was not detected by gas chromatography flame ionization detection in the rodent chow diet, while a trace amount of DHA was detected. Gas chromatography with mass spectrometry, however, was not able to detect any DHA in the rodent chow diet.

The safflower diet contained 13.3% oleic acid, 67.3% linoleic acid and 0.2% α-linolenic acid as a percent of total fatty acids. Similar to rodent chow, a trace amount of DHA was measured by gas chromatography flame ionization detection. Gas chromatography with mass spectrometry confirmed that the DHA percent composition was 0.01%. The trace amount of EPA is also believed to be artifact, although it was not confirmed by gas chromatography with mass spectrometry.

The fish oil diet was composed of 5.0% oleic acid, 58.8% linoleic acid, and 0.43% α-linolenic acid as a percent of total fatty acids. It was composed of 1.5% and 1.4% EPA and DHA respectively.