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Review

PPAR-δ and erucic acid in multiple sclerosis and Alzheimer's Disease. Likely benefits in terms of immunity and metabolism

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ABSTRACT

The transcription factor, PPARδ is involved in suppressing inflammation, stimulating oligodendroglial biogenesis and myelination. Furthermore, activation of PPARδ directly protects mitochondria against noxious stimuli and stimulates biogenesis of new mitochondria. PPARδ activation directly inhibits neuronal cell death and reduces both the level and neurotoxicity of Amyloid-β fibers in Alzheimer's Disease (AD) models. Among the important ligands of PPARδ is erucic acid (EA, 22:1 n9), an edible omega-9 fatty acid and a component of Lorenzo's oil, which is used in the treatment of adrenoleukodystrophy (ALD). Nonetheless, the feature of PPARδ-erucic acid interaction has not been extensively studied. EA can also be converted to nervonic acid, an important component of myelin. Hence, EA may act as an anti-inflammatory and remyelinating agent, which might be important in the management of another demyelinating disease, multiple sclerosis (MS). Oxidative injury and mitochondrial damage are among the features of ALD. Direct inhibitory effects of EA was observed on lipid peroxidation and inflammatory enzymes, neutrophil elastase and thrombin. EA also induces catalase, a potent antioxidant peroxisomal enzyme. However, EA is claimed to be a cardiotoxic molecule, yet these studies were mostly performed on rats, which do not efficiently metabolize EA. Further, EA is largely consumed by Asian population and Greenland Eskimos with no signs of cardiac damage. In this review, we shed light on the potential theraputic role of EA in MS and AD by blocking neural cell death, mitigating neuroinflammation and/or inducing myelination.

1. Introduction

Multiple sclerosis (MS) is a chronic autoimmune/neuroinflammatory disorder and the most common cause of neurological disability in young adults (after neurotrauma in some countries) [1]. First symptoms manifest in early adulthood, and a high percent of these patients develop physical disability and need help with walking within 15 years of disease onset? [1]. As the disease progresses, patients exhibit other symptoms including cognitive deterioration [1]. Current medicines reduce the inflammatory episodes of MS that contribute to physical disability, but they are not completely capable of inhibiting disease progression and the ongoing neuronal injury. Therefore, identifying novel pathogenic mechanisms and subsequent drugs altering such mechanisms is critical in MS. Dementia is a mental disorder manifested by impaired memory functions and deteriorated cognitive abilities, hindering the occupational and social activities of the individual [2]. Different clinical forms of dementia such as Alzheimer's Disease (AD), Lewy body dementia, frontal lobe dementia, Parkinson's disease, and cerebrovascular dementia are caused by progressive neuronal injury and are accompanied by cognitive decline [2]. The precise etiologies of these clinically distinct forms of dementia are not fully elucidated, although among these, the dementing condition that accounts for 50–60% of all cases is Alzheimer's Disease [2]. AD has affected > 37 million people worldwide, and the economic burden in USA alone is around US $100 billion [2]. Current medicines may slightly improve cognitive functioning in AD, yet no treatment is available to hinder disease progression. In this review, we discuss the roles of the transcription factor PPARδ and its ligand erucic acid (EA, a monounsaturated ω9-fatty acid denoted 22:1ω9) specifically with regard to their possible beneficial effects in MS and AD. EA is being used in the treatment of adrenoleukodystrophy (ALD) and it is an edible oil, largely consumed in some Asian countries and by Greenland Eskimos with no signs of toxicity. To demonstrate the tremendous potential of PPARδ-activation and EA in MS and AD, we performed a rigorous literatüre search and assimilated the data into specific sections in this review, including the presumable beneficial mechanisms of PPARδ in MS and AD (Fig. 1), the likely beneficial mechanisms of EA in MS (Fig. 2) and the potential beneficial mechanisms of EA in AD (Fig. 3).

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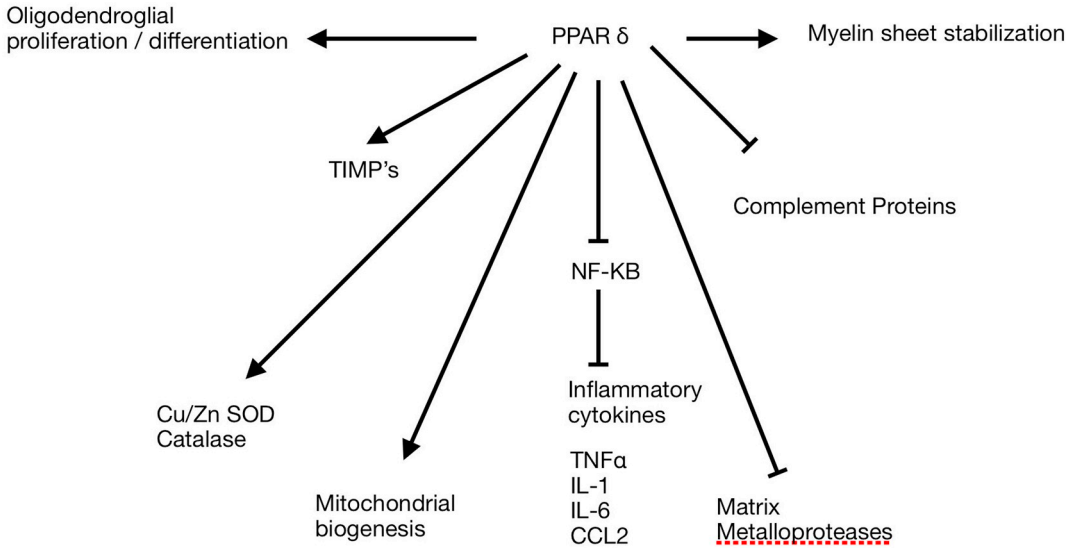

Fig. 1. The presumable beneficial mechanisms of PPARδ in MS and AD.

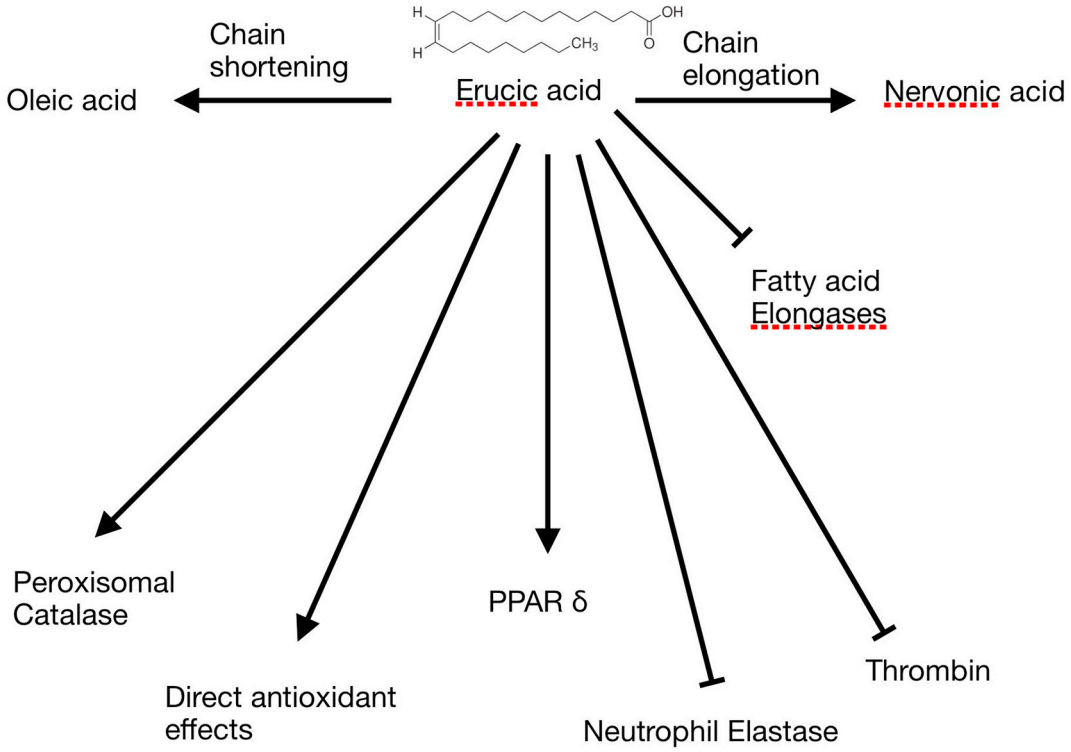

Fig. 2. The likely beneficial mechanisms of EA in MS.

All these actions were also summarized in Table 1.

2. Peroxisomes and peroxisome proliferator-activated receptors (PPARs)

Peroxisomes are organelles that perform a plethora of different metabolic functions including β-oxidation of fatty acids (FAs) and cholesterol metabolism [3]. Peroxisome proliferator activated receptors (PPARs) belong to a superfamily of steroid-thyroid hormone receptors which are stimulated by small lipophilic molecules including FAs and eicosanoids [3]. PPARs are nuclear receptor proteins that act as transcription factors regulating gene expression [4]. PPARs play crucial roles in the regulation of cell differentiation, embryogenesis and metabolism [5]. PPARs heterodimerize with the Retinoid X Receptor RXR and bind to specific DNA regions termed as PPREs (peroxisome proliferator hormone response elements). Activated PPARs regulate transcription through protein-protein interactions with other transcription factors including NFκB and STAT-1 and AP-1 signaling [3]. PPARs mainly consist of three subtypes; α, β/δ, and γ, each of which mediates the actions of different FAs and FA-derived molecules [3]. PPARs prominently modify energy metabolism; yet they differ in their activity [3]. PPAR-α is expressed mainly in the liver, and to a lesser extent, in muscle and heart tissues and is activated intrinsically by leukotriene B4 and extrinsically by lipid lowering drugs. PPARβ/δ is ubiquitously expressed, regulates energy expenditure and also in brain and oligodendroglial cells as will be detailed below [6,7]. PPARγ regulates energy storage and is expressed in endothelial and vascular smooth muscle cells and is further subdivided in four isoforms [3]. PPARγ is activated

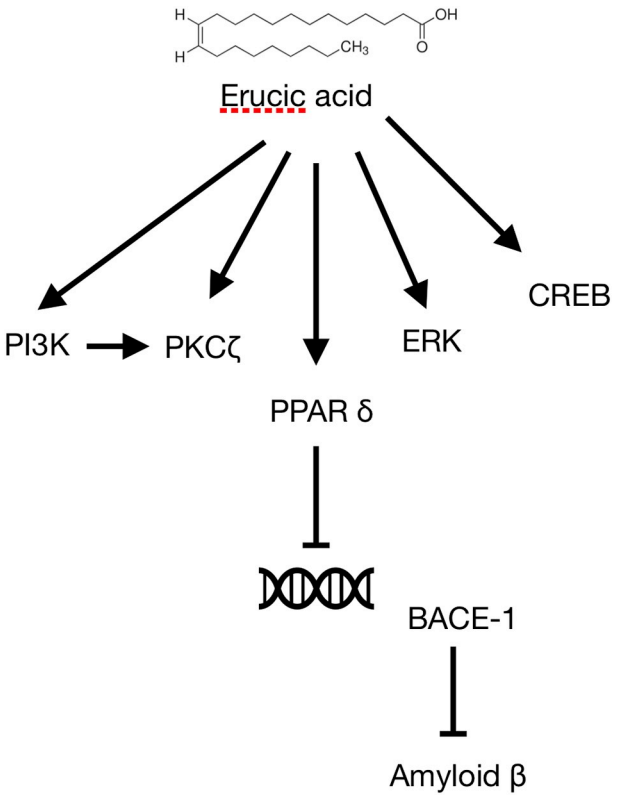

Fig. 3. Potential beneficial mechanisms of EA in AD.

Table 1
Potential mechanisms of putative EA benefits in MS and AD.

Effects of EA Relevant for Neuroprotection Both in MS and AD
Direct Antioxidant Effects
Stimulation of the Antioxidant Enzyme Catalase
Suppression of Elastase Which Degrades Extracellular Matrix and Activates Metalloproteases
Suppression of the Proinflammatory Thrombin Activity
Binding and activating PPARδ, which stimulates mitochondrial biogenesis
Effects of EA Relevant for Neuroprotection in MS
Direct Antiinflammatory Effects
Conversion (by chain shortening) to oleic acid, which potentiates EA effects in reducing VLCFA accumulation
Conversion (by chain elongation) to nervonic acid, a component of myelin
Direct Inhibition of fatty acid elongases and reduction of proinflammatory VLCFAs
Binding and activating PPARδ, which stimulates oligodendroglial proliferation, differentiation and myelin synthesis
Effects of EA Relevant for Neuroprotection in AD
Nootropic effects of rapeseed and mustard oils rich in EA
Direct nootropic effects of erucic acid via stimulation of PI3K/PKCζ, ERK and CREB pathways
Induction of PPARδ which suppresses activity of BACE-1, responsible of Amyloid β cleavage

by Prostaglandin J_2 and certain members of the 5-hydroxyeicosatetraenoic acid family of arachidonic acid metabolites including 5-oxo-15(*S*)-HETE and 5-oxo-ETE. Lastly, 15(*R*)-HETE, and 15-HpETE induce varying degrees of PPARα, β/δ, and γ.

3. MS Pathogenesis and general role of PPARs in MS

Multiple sclerosis (MS) is a demyelinating disease presented with chronic neuro-inflammation and breakdown of the blood–brain barrier (BBB) [8]. In cerebral and spinal regions, sustained inflammation causes destruction of the axonal myelin sheaths leading to neuronal death and the progressive loss of neurological functioning [8]. MS is considered an autoimmune disorder, presumably caused by diverse and interacting genetic and environmental factors. A specific group of glial cells, oligodendrocytes (OLs), produce myelin and wrap axons with multilamellar plasma membrane sheets. The loss of OL's and subsequently myelin plays a significant role in MS pathophysiology [8]. PPARs and the WNT/β-catenin pathways have opposing effects in many pathologic conditions, including MS. CanonicalWNT/β-catenin pathway is named after the discovery of the cascade gene “W”ingless in drosophila and its homologue in mice “INT”(Integration site). WNT ligands are glycoproteins regulating cell cycle and embryogenesis [8]. The dominance of the WNT/β-catenin pathway over PPAR pathway modifies energy metabolism leading to aerobic glycolysis and enhanced lactate levels, which further propagates neuro-inflammation. In parallel, there exists several data suggesting suppression of PPARs in MS and its protective role against MS pathogenesis: i) In EAE models, treatment with PPARγ agonists reduces inflammation and allows the remyelination in OLs; ii) PPARγ reduces the release of proinflammatory cytokines, and limits the expansion of encephalitogenic Th1, Th17 and B lymphocytes [8]. Putatively, PPARγ agonists may correct the unbalanced PPARγ versus WNT/β-catenin pathways, reduce anaerobic glycolysis and lactate production and alleviate neuro-inflammation [8]. As will be explained below, PPARδ activation stimulates mitochondrial biogenesis; and hence it is conceivable that, just like PPAR γ agonists, the detrimental shift to aerobic glycolysis may also be prevented by PPARδ agonists.

4. PPARδ: oligodendroglial differentiation, myelination and actions on experimental allergic encephalomyelitis

Central nervous system (CNS) accounts for only 2.1% of the body weight but it contains 23% of the total sterols [9]. In PPARδ-null mice, the total cholesterol content of the brain is not affected, however the brain cholesterol metabolism is perturbed [9]. Cholesterol in the CNS originates almost completely from in situ synthesis, whereas cholesterol can leave the brain and enter the blood in the form of 24-hydroxycholesterol [9]. Recycling of brain cholesterol is provided by cellular exchange through intermediate binding to apolipoproteins E and A1 [9]. Changes in the cholesterol metabolism in the whole body influences sterol recycling and expression of apolipoprotein E within the CNS. Brain lipid content of PPARδ-null female mice is affected with a 24% and 17% increase in phosphatidylethanolamine and phosphatidylserine, respectively, and a 9% decrease in the level of phosphatidylinositol [9].

Oligodendrocytes are major lipid-synthesizing cells whose regulation of lipid synthesis is essential to their activity as insulators of axonal conduction [10]. Expression of PPARδ is considerably higher in the developing neural tube and PPARδ knockout mice have abnormal myelination of the corpus callosum [6]. PPARδ is expressed in the mouse brain, with particularly high levels in the entorhinal cortex, hypothalamus and hippocampus [7]. PPAR-δ is expressed in oligodendrocytes and neurons but not in astrocytes [7,10]. PPARδ is prominently expressed in immature oligodendrocytes, indicating a role in oligodendrocyte differentiation [10]. The pattern of PPARδ expression matches closely to that of the myelin transcription factor I (MyTI), a zinc-finger DNA-binding protein that is expressed at higher levels in oligodendrocyte progenitors than in mature oligodendrocytes [10]. Partial and even complete agenesis of the corpus callosum may occur in fetal alcohol syndrome wherein ethanol induces abnormal myelination by hindering the synthesis of myelin lipids in oligodendrocytes [11]. In parallel, ethanol treatment of glial cells induces a concentration- and time dependent decrease in the levels of PPARδ, with no effect on PPAR-α or PPAR-γ [11]. PPARδ controls cerebral acyl-CoA synthetase-2 (ACS2) expression, which activates fatty acids to be converted into acyl-CoA derivatives for their metabolism [12]. Acylation is also a common modification of myelin proteins including proteolipid protein (PLP), which is crucial for the myelin sheath?-stabilization [12].

Primary glial cultures and enriched oligodendrocyte cultures of

neonatal mouse cerebra were exposed to three different PPAR agonists: a PPARγ-selective agonist, a PPARδ-selective agonist, and a pan agonist selective for both PPARγ and PPARδ [13]. Exposure to PPARγ agonist did not influence the differentiation of oligodendrocytes; however, PPARδ agonist and the pan agonist induced the differentiation of oligodendrocytes within 24 h of addition in mixed glial culture [13]. The number of oligodendrocytes with processes and huge myelin sheaths increased two- to threefold in both groups [13]. PPARδ agonist-treated groups contained more oligodendrocytes that expressed myelin basic protein (MBP) and PLP extending into distal processes [13].

PPARδ expression in naïve and injured spinal cords from adult rats was investigated in a spinal contusion model [14]. PPARδ was expressed by neurons and oligodendrocytes in uninjured spinal cords and also in NG2 cells (oligodendrocyte progenitors) within the white and gray matter [14]. After spinal cord injury, PPARδ$^+$ cell numbers decreased at 1 day post injury (dpi), likely due to neuronal loss, and then enhanced and became significantly higher than naive by 7 dpi and remained at high levels through at least 28 dpi. More than 20% of oligodendrocytes expressed PPARδ after spinal cord injury compared with ~10% in uninjured tissue. Further, the most prominent increase in PPAR-δ$^+$ oligodendrocytes was along the lesion borders where a portion of newly generated oligodendrocytes (BrdU$^+$) were PPARδ$^+$ [14]. The early rise in PPAR-δ$^+$ NG2 cells followed by an increase in PPAR-δ$^+$ oligodendrocytes indicates that this transcription factor may trigger both the proliferation and differentiation of oligodendroglia along spinal lesion [14]. In MS, premyelinating oligodendrocytes fail to myelinate axons [12], which maybe associated with decreased PPARδ expression in OL's due to enhanced inflammatory cytokines. In EAE (experimental autoimmune encephalomyelitis), agonists of PPARs alleviate neuronal damage [15]. In contrast to PPARγ, agonists of PPARδ were more efficient when given at later stages of EAE and increased myelin gene expression indicating stimulation of oligodendrocyte maturation [15]. In oligodendrocyte progenitor cells derived from E13 (embryonic day 13) mice, PPARd agonist, GW0742, but not a PPARγ agonist, increased the number of oligodendrocytes and production of myelin sheath [15]. Further, GW0742 alleviated cortical lesions, neuronal damage and glial inflammation [15].

MS involves CNS infiltration of myelin-reactive lymphocytes and macrophages, which typically accumulate in the perivascular regions and the parenchyma near terminal ovoids of the plucked axons [16]. Activated macrophages and microglia internalize myelin and secrete inflammatory mediators, which perturb axonal and myelin integrity [16]. Macrophages exert divergent phenotypes depending on their microenvironment and develop an inflammatory phenotype in MS. However, upon internalization of myelin, they may also become anti-inflammatory [16]. Myelin-derived cholesterol triggers this anti-inflammatory phenotype in myelin-phagocytosing macrophages by activating the sterol sensing liver-X-receptors (LXRs) [16]. Phosphatidylserine is an abundant myelin phospholipid that can also trigger phagocytosis of apoptotic cell debris by neighbouring cells. When phosphatidylserine flips to the outer membrane leaflet, it serves as a signal for phagocytosis [16].

Apoptotic cell clearance via phosphatidylserine shifts macrophages and microglia towards an anti-inflammatory phenotype, similar to myelin-phagocytosing macrophages [16]. Myelin as well as phosphatidylserine activate PPARδ in macrophages. Uptake of phosphatidylserine by macrophages, after intravenous injection of phosphatidylserine-containing liposomes (PSLs), blocks the synthesis of inflammatory mediators, reduces CNS-infiltration of immunocytes and ameliorates EAE [16]. PSLs also decrease cognate antigen-specific proliferation of splenic lymphocytes; PSLs are first internalized by splenic CD68$^+$ red pulp and CD169$^+$ marginal zone macrophages and both prevent and treat EAE. PPARδ is induced in foamy macrophages in MS lesions, which is proposed to be responsible for shifting macrophages towards an anti-inflammatory phenotype. It is also suggested that myelin-mediated PPARδ activation may help in scavenging myelin debris, which would inhibit oligodendrocyte maturation and axonal regeneration, thereby stimulating repair [16].

12/15-Lipoxygenase (12/15-LO) belongs to an enzyme family which metabolizes arachidonic acid to hydroxides such as 12-hydroxyeicosatetraenoic acid (12-HETE) and 15-HETE, which might be involved in inflammatory responses [17]. 12/15-LO is widely expressed in the CNS and is involved in various neurological diseases, including AD. Inhibition of 12/15-LO by baicalein significantly alleviates clinical severity of EAE [17]. Reduced migration of autoimmune T cells into the CNS by baicalein treatment was linked to reduced activation of microglia, which was demonstrated by their decreased synthesis of proinflammatory cytokines and chemokines. Baicalein also increased expression of PPARδ in microglia of EAE mice and is associated with reduced NF-κB and MAPK signaling [17]. Further, the induction of PPARδ in microglia decreased the expression of chemokines CCL2, CCL3, CCL20 and CXCL10 and alleviated CNS inflammation [17].

5. PPAR's and AD

All the three subtypes of PPAR are expressed different levels in the hippocampus. is expressed ubiquitously, whereas PPARδ localizes in the dentate gyrus/CA1 region, the expression of PPARγ is restricted to CA3 region [2]. One of the major features of AD is the cerebral accumulation of soluble β-amyloid (Aβ) peptides and the deposition of Aβ-fibrillar forms in extracellular compartments [18]. Isocortical areas are the first brain regions where Aβ-containing plaques deposit and then in limbic and allocortical structures such as entorhinal cortex and hippocampus [19]. The failure to clear Aβ deposits results in chronic production of toxic molecules which enhance AD pathology and neuronal death [18]. The accumulation of fibrillar Aβ-containing plaques causes the inflammatory activation of microglia and astrocytes juxtaposing these deposits [18]. Inflammatory cytokines in turn induce amyloid precursor protein (APP)-processing enzyme β-site APP cleaving enzyme-1 (BACE-1), leading to enhanced Aβ secretion. A PPAR-response element is present in the promoter region of BACE-1 gene, and the binding of PPAR to this response element suppresses the BACE-1 expression and subsequent Aβ-production [2]. Further, PPAR induction in glia and neurons leads to fast clearance and uptake of Aβ. AD patients exert perturbed cerebral glucose metabolism in several brain regions and the number and functioning of the neuronal mitochondria are disturbed [2]. Rosiglitazone, a PPARγ agonist, enhanced neuronal mitochondrial biogenesis, augmented glucose utilization and improved cognitive functioning in AD [2]. The induction of mitochondrial biogenesis was associated with the upregulation of mitochondrial DNA through stimulation of PPARγ coactivator-1 (PGC-1) family of proteins [2].

6. Particular relevance of PPARδ in AD pathogenesis

One of the major components of neuronal injury in AD is chronic activation of microglial cells around the plaques and their secretion of neurotoxic molecules [18]. On the other hand, a shift in microglial activation towards a phagocytic phenotype was proposed to be beneficial in AD by providing clearance of Aβ-deposits [18]. 5XFAD mice (a transgenic mouse model of AD) treated with the PPARδ agonist GW0742 for 2 weeks exhibited a reduction in the parenchymal Aβ load without affecting intraneuronal Aβ [18]. A significant decline in Aβ deposits in hippocampus and in cortical layer V was also observed. While a decrease in proinflammatory mediators (IL-1, IL-6, TNF-α and CCL2 chemokine) was observed, microglial reactivity surrounding Aβ deposits was enhanced [18]. AD brain has elevated expression of complement proteins and Aβ can directly activate the complement pathway [18]. Increased expression of these proteins also leads to neuronal degeneration injury; and the increased levels of complement proteins C3 and C1qa in 5XFAD mice were also attenuated with PPARδ agonist GW0742. Of note, the decrease in the inflammatory milieu

provided by GW0742 treatment led to alleviation of neuronal loss in the subiculum of 5XFAD mice [18]. The actions of GW0742, a selective PPARδ agonist on Aβ1–42-induced neurotoxicity was also analyzed in the hippocampus of mice [19]. Intra-hippocampal infusion of aggregated Aβ1–42 oligomer (410 pmol/mouse) prominently perturbed learning and memory in the Morris water maze (MWM) and Y-maze tests, which was associated with decreased hippocampal expression of PPARδ [19]. Intra-hippocampal infusion of GW0742 significantly attenuated Aβ1–42-induced memory deficits in mice and reversed hippocampal PPARδ decrease. GW0742 also repressed Aβ1–42- triggered neuroinflammatory and apoptotic responses, including nuclear NF-κB p65, TNF-α, IL-1β and cleaved caspase-3. Lastly, GW0742 also enhanced the ratio of Bcl-2/Bax in the hippocampus [19].

7. PPARδ: protection of mitochondria, involvement with neuronal antioxidant defense and providing direct neuroprotection

PPARs contain highly conserved DNA binding domains, while their ligand binding domains are more divergent [13]. While PPAR-δ is ubiquitous in tissues, some tissues express relatively higher levels of it including the brain, adipose tissue, and skin [6]. Compared with PPARα, the PPARδ functioning seems more important in muscle and less so in the hepatic tissue [20]. Mice with a cardiomyocyte-restricted knockout of PPARδ suffer from progressive cardiomyopathy with lipid deposition, mitochondrial damage and a shift from fatty acid towards glucose utilization in the myocard [20]. Conversely, a constitutively active cardiac PPARδ in transgenic mice increased mitochondrial biogenesis and antioxidant defense, such as PPAR-γ coactivator-1 besides the Cu/Zn superoxide dismutase, catalase and fatty acid metabolism-enzymes, including carnitine palmitoyltransferase Ib and carnitine palmitoyltransferase II [21]. Subsequently, short-term PPARδ knockout in the adult mouse heart decreased both the Cu/Zn- and manganese-dependent forms of superoxide dismutase and enhanced oxidative damage in myocardial tissue [22]. In primary embryonic cortical neuron culture, PPARδ resides in the nuclei of neurons and enhances in relation to their maturation, in accompaniment with its heterodimer partners RXR-β and γ. The associated induction of the PPARδ target gene ACS2 indicates that PPARδ is active [9]. The role of PPARδ in cerebral repair was first investigated in a focal cerebral ischemia model [9]. Compared with wild type, PPARδ-null mice demonstrated a significant increase in the infarct area, revealing that PPARδ has a neuroprotective function. Of note, the difference in infarct area between wild-type and PPARδ-mutant mice was witnessed as early as 30 min following the ischemia, indicating that PPARδ could function in the very early steps of neuronal injury [9]. Reciprocally, in a transient middle cerebral artery occlusion, intracerebral infusion of PPARδ agonists in rat brain ventricle significantly decreased the brain infarct area 24 h after reperfusion [9]. Furthermore, in a similar ischemic stroke model, an enhancement of malondialdehyde and a decline of glutathione and manganese superoxide dismutase in PPARδ-null mice indicate its neuroprotective role against oxidant stress [9]. Lastly, it was shown that activation of PPARδ by adenoviral gene therapy or by treatment with a PPARδ-agonist significantly reduced brain injury with improvement in neurological deficits, brain edema, blood–brain barrier injury, and with prominent decrease in neuronal cell apoptosis at 24 h after subarachnoid hemorrhage in rats [23]. Further, inflammatory transcription factor NFκB and matrix metalloprotease-9 (MMP9) activation following hemorrhagic stroke was also inhibited by activation of PPARδ [23].

8. PPARδ and mitochondrial biogenesis

In the cardiac tissue, the 3 subtypes of PPARs, PPARα, δ, and γ, are present at various levels and regulate lipid and glucose metabolism [22]. Cardiac PPARδ is required for appropriate expression of fatty acid-oxidation enzymes. The heart mainly uses glucose to produce ATP in a newborn animal and switches to use long-chain fatty acids after weaning, which is accompanied with transcriptional enhancement of fatty acid oxidation genes [22]. Mitochondrial biogenesis inducers such as PGC-1α and PGC-1β were significantly decreased in the short-term PPARδ deficient heart, concomitant with a declined mitochondrial DNA copy number [22]. Mitochondrial biogenesis is the growth and division of preexisting mitochondria, which is a continuous renewal phenomenon necessary for the cells to handle challenges in energy demand [22]. PPARδ directly regulates the expression of PGC-1α and is involved in the transcriptional control of mitochondrial biogenesis in the skeletal muscle [22]. Mitochondrial deficiency due to the decreased mitochondrial biogenesis contributes to the decline in palmitate and glucose oxidation in the cardiomyocyte restricted PPARδ-knockout mice [22]. Prior to embryo implantation in vivo, embryonic cells localize in a hypoxic micro-environment and depend on anaerobic metabolism [24]. PPARδ is also involved in embryonic implantation and PPARδ knockout embryos have placental abnormalities [24]. Embryonic stem cells have very few and small mitochondria with simple cristae, whereas differentiated cells develop a normal mitochondrial morphology with an accompanied enhancement in aerobic ATP production [24]. Maturation of hepatic tissue from embryonic stem cells and mitochondrial biogenesis is induced by a PPARδ specific agonist and inhibited by a PPARδ specific antagonist, and is unaffected by PPARγ specific agonists [24].

Recent evidence further links PPARδ activation to mitochondrial biogenesis. 24-h leucine treatment of cultured myotubes significantly enhanced PPARδ expression as well as markers of mitochondrial biogenesis, leading to significantly enhanced mitochondrial content and oxidative metabolism [25]. However, leucine-treated cells did not show significant changes in uncoupling protein expression or oxygen consumed per relative mitochondrial content indicating that leucine-induction of oxidative metabolism is a function of increased mitochondrial biogenesis [25]. Conjugated linoleic acid (CLA) with exercise training increases endurance capacity via the PPARδ-mediated mechanism in mice [26]. CLA treatment significantly enhanced expression of sirtuin1 (SIRT1) an up-stream regulator of PPARδ, in both sedentary and trained animals [26]. With respect to downstream markers of PPARδ, CLA induced the key factors essential to induce mitochondrial biogenesis, Nuclear Respiratory Factor-1 (NRF-1) and mitochondrial Transcription factor A (Tfam) [26].

9. Erucic acid (EA) and neurodevelopment

In human brain development, the first stages of embryogenesis is characterized by prominent proliferation of neural stem and progenitor cells; while in later stages, cell differentiation events and formation of cell-to-cell contacts occur. During the last trimester, increased fatty acid chain elongation and desaturation within the infant brain and tissue leads to accretion of saturated and ω9 fatty acids and increase in whole brain weight [27]. Myelination of nerves in human brain starts at a gestational age of approximately 32 weeks, increases rapidly until and beyond birth, and is largely complete in the first 2 or 3 years of life [28]. Myelin sphingolipids contain a high proportion of their total fatty acids as very long chain saturated and especially very long chain monounsaturated moieties exemplified by lignoceric acid (24:0) and ω9 fatty acid nervonic acid (24:1ω9), respectively [28]. EA can be converted to nervonic acid by human hepatocytes [29] and binds to the transcription factor hNUC1/PPAR-δ [35]. PPAR-δ expression peaks during late brain development coinciding with the onset of myelination and is involved in the differentiation of oligodendroglial cells [14]. EA levels are prominently high in Chinese women milk [30] and Chinese infants develop less brain tumors in comparison to Western countries according to the IARC data (International Agency for Research on Cancer) [31]. EA may act as a differentiation agent for oligodendroglial/astrocytic biprogenitors and may also reduce the risk of neurocarcinogenesis.

10. Adrenoleukodystrophy and erucic acid content of Lorenzo's oil

Adrenoleukodystrophy (ALD) is caused by perturbed peroxisomal fatty acid β-oxidation, which cause the accumulation of very-long chain fatty acids (VLCFAs) in several organs, particularly the adrenal cortex, testis and the central nervous system (CNS) [32]. Myelinated tissues are most severely affected, causing diverse clinical symptoms, including vegetative state in children to paraparesis in adults [32]. ALD is caused by mutations in the X-linked ABCD1 gene, which encodes a peroxisomal membrane protein required for transporting VLCFAs into peroxisomes [32]. Mitochondria are main organelles of lipid catabolism, but VLCFAs (> 22 carbons) cannot be metabolized in mitochondria, and need to be transported into peroxisomes to be catabolized [32]. VLCFAs belong to two different lipid types, glycerolipids and sphingolipids, which have different lipid backbones, a glycerol backbone in the former and a sphingoid base in the latter [33].

Lorenzo's oil therapy, which consists of a 4:1 mixture of glycerol trioleate and glycerol trierucate, is the only partially effective treatment for ALD patients [34]. After oral intake, the triglycerides are hydrolyzed by lipases in the intestines to liberate EA and oleic acid, which are then absorbed and transported via lymph and blood to the tissues [33]. Lorenzo's oil reduces the abnormally high levels of circulating C24:0 and C26:0 in ALD patients; but, does not change the prognosis of patients with preexisting neurological dysfunction. However, it may slower disease progression in asymptomatic patients [33]. Oleic acid aims at reducing dietary saturated fatty acids and EA aims at lowering the elongation of docosanoic (22:0) to 26:0, by blocking the activity of microsomal elongases [34]. Recent studies also indicate that both oleic and EA may act via fatty acid elongation [33]. VLCFAs are produced by the fatty acid elongation cycle in the endoplasmic reticulum via addition of C2 units from malonyl-CoA to long-chain acyl-CoA [33]. Each elongation cycle includes four steps: condensation, reduction, dehydration, and reduction [33]. The first condensation reaction is the rate-limiting step catalyzed by ELOVL (Elongation of Very Long-chain fatty acid)-family of enzymes. Seven known ELOVL isozymes (ELOVL1–7) exist in mammals, which exert diverse specificity for chain length and degree of saturation of the acyl-CoA substrate [33]. ELOVL1 is selectively active on saturated and monounsaturated C20– to C24-CoAs and is involved in the synthesis of C22- to C26-VLCFAs such as those accumulated in ALD [33]. EA and oleic acids block the activity of ELOVL1; moreover, the FA composition of Lorenzo's oil (4:1 mixture) provides the most efficient inhibition [33]. At the cellular level, treatment with the 4:1 mixture decreased sphingomyelins with a saturated VLCFA accompanied by increased sphingomyelins with a monounsaturated VLCFA, likely due to the incorporation of EA acid into the fatty acid elongation cycle. These findings indicate that inhibition of ELOVL1 may be a potential mechanism by which Lorenzo's oil exerts its action [33]. A study demonstrating that EA binds selectively to PPAR-δ [35] has been overlooked, which might be useful for the management of other demyelinating and neurodegenerative disorders. Nonetheless, we should also underscore that FDA has not approved the use of Lorenzo's oil as a therapeutic drug for X-linked ALD. Lorenzo's oil lowers the VLCFA in postmortem adipose tissue and liver in ALD when compared with untreated ALD tissue. However, it is difficult to prove that Lorenzo's oil lowers the VLCFA in human ALD brain as one would need to study the fatty acid composition of lipids in intact myelin in Lorenzo's oil treated compared with untreated ALD myelin isolated from brain areas with normal appearing white matter. Lipid studies performed on active demyelinating areas in ALD brain may not reflect the real situation as there are fatty acid changes secondary to inflammation and myelin loss.

11. Erucic acid: chain shortening to oleic acid, chain elongation to nervonic acid and penetrance into the brain tissues

Although at low levels, there is evidence indicating that dietary EA could enter the brain. Golovko and Murphy investigated the ability of EA to cross the blood-brain barrier (BBB) by infusing ^{14}C 22:1n-9/EA (170 μCi/kg, iv (intravenous) and icv (intracerebroventricular)) into male rats [36]. ^{14}C-arachidonic acid (20:4n-6) iv was the positive control. After iv infusion, 0.011% of the plasma ^{14}C-22:1n-9/EA was detected in the brain, compared to 0.055% of the plasma ^{14}C-20:4n-6 [36]. The ^{14}C-22:1n-9/EA was highly β-oxidized (60%), compared with only 30% of ^{14}C-20:4n-6 [36]. 20:4n-6 incorporated mainly to phospholipid pools, EA incorporated to cholesteryl esters, triglycerides, and phospholipids [36]. When ^{14}C-22:1n-9/EA was infused into the fourth ventricle of the rat brain for 7 days, 60% of the EA was incorporated to the phospholipid pools, similar to that observed for ^{14}C-20:4n-6 [36]. This indicates the cerebral plasticity to esterify EA in an exposure-associated manner [36]. In i.v. and i.c.v. infused rats, significant levels of tracer found in the phospholipid pools underwent chain shortening and were detected as ^{12}-^{14}C-20:1n-9 eicosenoic and ^{14}C-oleic acid [36]. These findings revealed that intact EA crosses the BBB, is incorporated into lipid pools, and is chain-shortened. These studies did not find any changes in the nervonic acid (C24:1) levels in brain following EA administration and commented that chain elongation of EA does not occur in brains [36]. However, their treatment duration and observation period was very short and rats poorly metabolize EA. Indeed, previous rat studies showed chain elongation of EA to nervonic acid/24:1ω9 in brain. Feeding of rats with high doses of EA was also found to elevate both oleic and nervonic acids in plasma phospholipids, indicating both chain shortening and elongation, respectively [29]. Nonetheless, it should be noted that Louw did not measure brain fatty acids; therefore, this is not an evidence for EA conversion into nervonic acid in the brain. Cultured human fibroblasts convert EA into nervonic acid and cultured hepatocytes convert EA to C20:1ω9 (eicosenoic acid), C18:1ω9 (oleic acid) and C16:1ω9 [29]. Feeding rats with large doses of EA was also shown to enhance cerebral arachidonic acid levels which was attributed to a phenomenon that EA induces both protective and pro-inflammatory effects within the brain [29]. Nonetheless, the applied doses were too high (400–800 mg/kg) and the animals studied were rats [29], which are particularly vulnerable to EA toxicity.

12. Erucic acid for remyelination by being a direct source of nervonic acid

Myelin has unusual features among biological membranes and contains > 70% of its dry weight as lipids rich in sphingolipids, i.e. cerebrosides, sulphatides and sphingomyelin which constitute about 23%, 4% and 8% of total myelin lipids, respectively. These sphingolipids contain a high level of their total fatty acids as very long chain saturated and especially very long chain monounsaturated moieties exemplified by lignoceric acid 24:0, and nervonic acid 24:1ω9, respectively [28]. The level of nervonic acid in human brain sphingolipids increases markedly from birth to reach a maximum at about 4 years after which it remains almost constant [28]. Given such a large demand for relatively specialised fatty acids over a relatively short and critical period of neuronal development, an impaired provision of these fatty acids early in life could have serious consequences for neuronal performance, whether in the short or long term [28]. Nervonic acid normally accounts for around 40% of the total fatty acids in sphingolipids from the brains of normal subjects [28]. However, in ALD brains, the nervonic acid in sphingomyelin is decreased to < 30%; and also in postmortem brain samples of MS, a marked decrease of nervonic acid in sphingomyelin, sulphatides and cerebrosides is witnessed which is almost exactly balanced by an increase in 18:0 + 16:0 fatty acids [28]. The decreased nervonic acid in brain sphingolipids in MS may be an effect of demyelination rather than being a consequence of disease process [28].

Nonetheless, an association between impaired biosynthesis of nervonic acid with demyelination is strongly supported with the observations that the mutant ‘jumpy’ and ‘quaking’ mice suffer from extensive

demyelination which both have defects in the microsomal fatty acid elongases responsible for VLCF-biosynthesis including nervonic acid [28]. The results of such an impairment will be most damaging during the early stages of brain development when myelination is extensive and when the myelin levels of nervonic acid and 18:0 are increasing and decreasing, respectively [28]. Nonetheless, demyelination due to perturbed nervonic acid synthesis may not necessarily be limited to early brain development, especially if the impairment is partial and leads to unstable myelin conformations. Thus, partially reduced nervonic acid synthesis could generate myelin with a reduced stability, causing an incomplete compaction as occurs in the 'trembler' mouse [28]. Such a myelin would then have an increased vulnerability to environmental noxious events at any subsequent stage of life, causing it to be stripped from nerves and stimulating autoimmune responses [28]. As suggested above, EA can be converted to nervonic acid by human hepatocytes [29]. Further, increasing the concentration of very long chain monoenes including EA inhibits the biosynthesis of 26:0 from its 18:0 precursor and simultaneously enhances the biosynthesis of nervonic acid as evidenced by the enhanced levels of serum nervonic acid in ALD patients treated with Lorenzo's oil [28]. Increased nervonic acid in erythrocyte sphingomyelin is also witnessed in normal subjects fed borage oil rich in EA [28]. Borage oil contains EA and nervonic acid in low amounts; and notably, it is used in the treatment of MS in folkloric medicine in Mediterranean countries [37]. Overall, there exists the possibility that EA may alleviate MS via enhancing the levels of nervonic acid, which is an important component of myelin.

13. Adrenoleukodystrophy association with inflammation and neuroimmune pathways. Similarities and dissimilarities to MS

The first brain autopsy in an ALD-effected child in 1913 revealed an accumulation of lymphocytes, lipid laden macrophages and glial cells, which resembled MS and was termed as "encephalitis periaxialis diffusa" [38]. Peculiarly, not all males with ALD suffer from CNS demyelination and related neuroinflammation, indicating that other factors (genetic, epigenetic and environmental) are involved in the CNS-pathogenesis of ALD [32]. Likewise, in mice knock out for the ABCD1 gene, VLCFA-accumulation occurs, yet only mild neurological signs of ALD develop [34]. A striking inflammation in ALD is unique in comparison to other leukodystrophies [29] and the significant amount of inflammatory cells observed in ALD is also not seen in other known cerebral metabolic disorders [38]. Cerebral inflammation develops in < 50% of the patients with the ALD gene defect and there is no way of predicting which patients will develop the fatal inflammatory phase [29]. The nonenzymatic free radical products eicosanoids (including isoprostanes) are catabolized by β-oxidation in peroxisomes and hence, a decrease in peroxisomal β-oxidation may lead to accumulation of inflammatory eicosanoids and lipid peroxidation products [34]. Here, it should be noted that EA increases peroxisomal content [39] and peroxisomal catalase activity [60] in cardiac muscle. All the cerebral forms of ALD are inflammatory forms, associated with a fast and progressive inflammatory myelinopathy, that mostly begins in the parietooccipital regions and may involve autoimmune mechanisms, as was first suggested by Adams and Kubic [34,38,40]. Inflammatory myelinopathy is especially present in the fast- progressive childhood form with perivascular infiltration of lymphocytes and macrophages to the brain similar to that seen in MS [38].

In 1983, Bernheimer et al. investigated the presence of immunoglobulins in ALD and MS brains, while emphasizing that the prominent inflammation in ALD is not merely due to lipid storage or tissue injury [69]. Immunoglobulins (Igs) were analyzed in cerebral white matter of two ALD patients, three MS patients, and three controls. In ALD brains, free IgG and IgA were increased, and IgM was also increased in one sample, compared to controls [69]. Increased free IgG and IgA were also seen in MS brains; but in contrast to ALD and controls, significant levels of bound Igs (IgG) extractable at acid or alkaline pH, were also present in MS brains. The authors have commented that the lack of Igs extractable at acid or alkaline pHs in ALD brains did not necessarily mean the absence of antibodies against ALD brain antigens, since bound Igs might have been extracted as soluble immune complexes in the pH 7.4 fraction [69]. Additionally, immune cytochemical studies revealed accumulation of IgG, IgA and IgM-positive lymphoid cells. Specifically, IgG positive cells were more abundant than IgA and IgM positive cells, and some areas were prominently rich in IgA positive cells [69]. Likewise, vascular binding of IgA was witnessed in ALD brains, but not in MS brain. This difference is not due to different Ig levels, because IgA concentrations in the ALD and MS extracts were in the same order of magnitude [69].

Griffin et al. have immunotyped the perivascular cuff cells in ALD brains and found that 59% were T cells, 24% B cells, and 11% monocytes and macrophages [38,41]. Boutin et al. characterized the macrophages in frozen brain specimens from an ALD-patient by immunohistochemical staining [68]. This study showed that macrophages were the main cells in the white matter with a wide variability in their subtypes according to their location. In the perivascular cuffs, the cells were positive for CD11b, CD11c, CD14, CD35 and CD68, while in the white matter, they stained mainly with CD11c and CD68 and lesser with CD11b and CD35 [68]. In addition to MHC class II and CD4, macrophages were also IL-2 Receptor positive [68]. Powers et al. studied immune infiltrations in 25 demyelinating lesions from 5 juvenile ALD, 3 adult ALD, and 3 adrenomyeloneuropathy cases [42]. Macrophages and astrocytes were the main cells in the active edge; T lymphocytes, including CD4 and CD45R subsets, were nearly as numerous and usually located perivascularly [42]. B cells and IgG-positive plasma cells were infrequent. MHC-I expression was enhanced in vascular and other cells. Myelin debris expressed C3d and IL-1; IL-1 and ICAM-1 expression was seen on microvessels and astrocytes [42]. TNF-α immunoreactivity was witnessed in macrophages, but higher in astrocytes [42]. Ito et al. also investigated immunocytes in the early white matter lesions of ALD. Most lymphocytes were CD8 cytotoxic T cells which infiltrated morphologically unaffected white matter [43]. MHC class II- and TGF-β-positive microglia were also found. CD44, which can mediate MHC-unrestricted cell death, was seen on many lymphocytes and white matter elements. CD1 molecules, which play main roles in MHC-unrestricted lipid antigen presentation, were also detected [43]. The authors indicated that CD8 cytotoxic T cells maybe involved in early stages of oligodendrocyte cell death [43].

As suggested above, the accumulation of VLCFA in different tissues, including the brain is the major cause of ALD [34]. An increase of 26:0 incorporated into the cell membranes may trigger both the demyelination and subsequent enhancement of antigenicity [34]. It was also proposed that the abnormal VLCFA-accumulation may cause the release of lipids which induces a cytokine storm similar to that caused by lipopolysaccharide [38]. In ALD, excess VLCFA is already present in the foetus and precedes the manifestation of neuropathology. Excessive VLCFA occurs in all brain lipids, the greatest in the ganglioside, phosphatidylcholine, proteolipid and cholesterol ester fractions. Each of these could be involved in stimulating inflammation, with gangliosides a particularly plausible candidate [38]. Abnormally high VLCFA content in myelin proteolipid from ALD brain was also shown, an observation that is of interest since antibodies to PLP are implicated in the development of MS [38].

Due to the previous evidence which showed the presence of B cells in perivascular cuff and complement factors in degenerating myelin in ALD, Schmidt et al. analyzed the sera of 51 patients with various phenotypes of ALD, 20 patients with MS and 22 healthy volunteers for the presence of autoantibodies against human myelin oligodendrocyte glycoprotein $rhMOG^{IgD}$ and myelin basic protein (MBP) [70]. Anti-$rhMOG^{IgD}$ autoantibodies were significantly more frequent in ALD and MS patients compared to healthy individuals ($p < 0.05$). Anti-MBP autoantibodies were present in about one-fourth of ALD and MS patients but in < 10% of healthy individuals. Noteworthy, $rhMOG^{IgD}$

antibodies were shown in 3 of 6 patients with the cerebral ALD while there were absent in any of the 13 patients with adrenomyeloneuropathy, a slowly progressive peripheral myelopathy with little or no inflammation. The authors pointed to undisputable similarities between MS and ALD, such as the increase of inflammatory cytokines including TNF-α and IFN-γ on macrophages, astrocytes and endothelial cells as demonstrated by previous studies [70]. Besides the noteworthy similarities in inflammatory components of ALD and MS, several differences were also noted. Oligoclonal bands in the cerebrospinal fluid are present in only a small proportion of ALD patients, but they exist in 90% of MS patients [38]. The association between MS and HLA-DR2 haplotypes were documented repeatedly which does not exist in cerebral ALD [44]. It was also suggested that the expression of inflammatory cytokines in MS was more intense than in ALD [38,44]. Inflammatory cytokines such as TNF-α decreases VLCFA oxidation and plasmalogen synthesis via inhibiting the expression of PPAR-δ [12], which is a transcription factor binding EA [35]. TNF-α also activates sphingomyelinase cascade with the production of ceramide, which triggers apoptosis in several cell types including oligodendrocytes [12]. Hence, TNF-α and abnormally accumulating VLCFA may trigger a positive feedback loop leading to neural tissue injury and myelin degradation. Below, we will provide studies to suggest that EA may alleviate inflammation, and consequently might be associated with therapeutic effects in ALD. If one of the main mechanisms of EA action is suppression of inflammation, it is also possible such an anti-inflammatory effect would also be beneficial in MS.

14. Erucic acid and erucic-acid rich oils. Direct anti-inflammatory effects

Despite EA being employed in the treatment of ALD, a disease with prominent neuroinflammatory components, very sparse data exists on the effects of EA and EA-rich oils on the immune system. Therefore, opposite findings exist regarding actions of EA and EA-rich oils. Nonetheless, it would be proper to outline these immune effects. Unkrig et al. studied 15 men with ALD and 3 symptomatic heterozygous women. The patients received a low-fat diet and a mean of 1.28 g glycerol trioleate and 0.23 g of glycerol trierucate [45]. Among these 18 patients, 5 patients developed asymptomatic thrombocytopenia and lymphocytopenia (mean 400 per cubic millimeter range 10 to 670, $p < 0.0025$) [45]. The total white cell counts, absolute number of neutrophils monocyte, and levels of immunoglobulins (IgM, IgG, IgA and IgE) were normal [45]. Flow cytometry revealed no relative decrease in T cells, B cells or Natural Killer cells and CD4:CD8 ratio was also normal [45]. No cytotoxic anti-lymphocyte antibodies were found. Two of these patients developed recurrent infections [45]. These results are contradictory to the research data of Pour et al. who investigated changes in immunity and lymphocyte proliferation in response to mitogens (PHA, Con A, PWM, OKT3). They compared immunity parameters in 27 ALD patients receiving Lorenzo's Oil and in 14 patients without treatment [46]. In patients on treatment, mitogen-stimulated lymphocyte proliferation was significantly enhanced in comparison to patients without treatment [46]. Lymphocyte proliferation in patients without treatment was comparable to that of normal controls. Additionally, increased concentrations of EA was found in lymphocytes from patients with treatment. Enhanced mitogen-responses were attributed to changes within the mononuclear cells themselves and not to changes in serum content, as addition of EA-rich plasma to untreated cells did not modify proliferative responses [46].

In 1993, Aubourg conducted an open trial of Lorenzo's Oil treatment in 14 men with ALD, 5 symptomatic heterozygous women and 5 boys with preclinical ALD [47]. They reported that 3 of these patients (12.5%) developed asymptomatic neutropenia (500 to 700 per cubic millimeter) [47]. This is again somewhat opposite to the findings of Heiskanen and Savolainen which showed stimulatory effects of EA on neutrophil functions; they investigated effects of EA on the oxidative burst of neutrophils [48]. Oxidative burst in neutrophils is associated with superoxide production and subsequent production of reactive oxygen species (ROS). This phenomenon may be induced by G-protein coupled receptor agonists or direct Protein Kinase-C (PKC) activators such as FMLP (formyl-Methionyl-Leucyl-Phenylalanine) and PMA (Phorbol Myristate Acetate), respectively [48]. PKC induces a multicomponent respiratory burst enzyme, NADPH oxidase, which catalyzes the reduction of oxygen to superoxide. EA did not alter the amount of intracellular calcium, but dose-dependently triggered respiratory burst in neutrophils [48]. EA-stimulated production of ROS was fast yet transient; it started instantly after treatment of the cells with EA, and the maximal production of ROS occurred within 4 min [48]. A PKC-inhibitor completely blocked EA-induced production of ROS.

Sedlmayer et al. analyzed three patients with ALD for the concentration and activity of natural killer (NK) cells in the peripheral blood [49]. Two patients were receiving glycerol trierucate during the whole period of testing; the third patient was off diet for 6 months prior to the tests [49]. Phenotyping of NK cells, using monoclonal antibodies against CD3, CD16 and CD56, revealed a slight decrease in the NK cells in one patient on diet in two out of three occasions. For ^{51}Cr-release cytotoxicity assays, K562 cells were used as targets. In both ALD patients on Lorenzo's oil, a severe decrease in NK-lytic activity on three occasions was witnessed during the course of 4 months [49]. The percentages of NK cells in relation to total lymphocytes and the absolute numbers of NK cells did not decline to a level that could explain the low NK activity. Further, during the time of observation no clinical symptoms were observed, which would develop due to impaired NK-function such as frequent viral infections [49]. Here, it should be noted that pure cell culture studies with EA showed more immuno-stimulating effects, while direct patient observations or investigations on cells obtained from patients indicated more immunosuppressive effects.

Proteolytic degradation of extracellular matrix is an important component of diverse physiological and pathological processes including embryogenesis, immune diseases and tumor metastasis [50]. Serine protease human neutrophil elastase (EC 3.4.21.37) is a major enzyme for the degradation of connective tissue. Elastase itselfs cleaves collagen types I, II, III, IV, VIII, IX, X and XI as well as elastin and other proteins with important biological functions [50]. Furthermore, elastase indirectly induces the destruction of matrix proteins via activating matrix metalloprotease-9 (MMP-9). There are also some studies that indicate elastin activates MMP-2. Rennert and Melzig investigated the actions of 17 free fatty acids (saturated and unsaturated, C_{12}-C_{22}) on elastase [50]. Among the tested 17 fatty acids, EA was the most effective inhibitory fatty acid on elastase with an IC_{50} value of 0.45 μM [50]. Considering the fact that EA is a ligand of PPAR-δ, EA-inhibition of elastase is similar to the effect of PPAR-δ agonist in preventing aortic aneurysm progression by blocking elastin, degrading collagen and activating MMP-2 [71]. These studies will be discussed below.

Thrombin is also a member of the chymotrypsin family of serine proteases similar to neutrophil elastase and is induced with the blood coagulation cascades [51]. Besides its activity on thrombosis, it can also induce inflammation via a specific protease activated receptor-1 (PAR-1) because proteolysis is required for its activation. Other PARs, PAR-2, -3 and -4 were also discovered, among which the PAR-3 and PAR-4 can also be activated by thrombin [51]. PARs exist in many tissues, and they are involved in several inflammatory diseases. Inhibition of thrombin activity inhibits PAR-1, -3 and -4 and their related inflammatory effects. Melzig and Henke studied the effect of 10 different fatty acids on the activity of thrombin and found that only oleic acid and EA could effectively inhibit thrombin [51]. Nonsaturated fatty acids with 18 carbon atoms inhibited thrombin enzyme activity stronger than saturated fatty acids (18:1 ω9 oleic acid $IC_{50} = 7$ μM versus 18:0 stearic acid $IC_{50} = 786$ μM), as well as fatty acids with 22 carbon atoms (22:1 ω9 EA $IC_{50} = 5$ μM versus 22:0 behenic acid $IC_{50} < 1000$ μM). EA was the most efficient inhibitor of thrombin among the 10 tested fatty acids [51]. Overall, EA may possess anti-

inflammatory functions due to its inherent molecular features, but it may also suppress inflammation by virtue of being a ligand of PPARδ, a transcription factor with anti-inflammatory effects detailed below.

15. PPARδ: effects on inflammation and immunity

In contrast to PPARα and PPARγ, expression of PPARδ is ubiquitous, with high levels in macrophages [52]. The anti-inflammatory features of PPARδ activation are associated with the release of B cell lymphoma 6 (bcl6) from the unliganded PPARδ, which hinders expression of Activator Protein-1 (AP-1)–inducible cytokines [52]. Further, in adipocytes and cardiomyocytes, PPARδ agonists block lipopolysaccharide-induced and NFκB-induced cytokine expression [52]. PPARδ activation could also directly trigger the physical binding between PPARδ and the p65 subunit of NFκB and decrease the degradation of the inhibitory protein IκB, thus preventing NF-κB activation [53]. THP-1 macrophages exposed to very low density lipoprotein (VLDL) demonstrated significant triglyceride accumulation, which was alleviated by PPARδ activation [52]. VLDL-treated macrophages significantly enhanced their expression of AP-1 and associated cytokines IL-1β, macrophage inflammatory protein 1α (MIP-1α) and intercellular adhesion molecule-1 (ICAM1) [52]. VLDL treatment increased the phosphorylation of both extracellular signal-related kinase-1 and -2 (Erk1 and Erk2) and p38. VLDL reduced AKT phosphorylation as well as its downstream effector forkhead box protein O1 (FOXO1), concomitant with increased FOXO1 [52]. Cells treated with PPARδ agonists become resistant to VLDL-triggered expression of inflammatory cytokines, mediated by normalization of MAPK/^{erk} and AKT/FOXO1 signaling [52]. Further, the efficiency of PPARδ agonists to alleviate the inflammatory response is independent of agonist-blockage of lipoprotein lipase, stimulation of β-oxidation, or decline in cellular triglycerides [52]. Mice fed with a high-fructose corn syrup (HFCS) diet develop hyperlipidemia, hyperinsulinemia, hyperleptinemia, and hypoadiponectinemia [53]. GW0742 improved glucose and lipid metabolism via PPARδ upregulation [53]. HFCS-induced activation of NFκB and ICAM-1was also alleviated by drug treatment. These effects were accompanied by a decline in the serum IL-6 levels and an enhancement in muscular FGF-21 (Fibroblast Growth Factor-21) expression [53].

Inflammation is involved in the formation of abdominal aortic aneurysm (AAA), characterized by a localized degradation of connective tissue and apoptosis of vascular smooth muscle cells [71]. Hwang et al. investigated whether the ligand-activated PPARδ can hinder angiotensin II (Ang II)-induced AAA formation in apoE-deficient mice [71]. GW501516, an activator of PPAR-δ, decreased both the incidence and the extent of Ang II-induced AAA in apoE-deficient mice [71]. Ligand-activated PPARδ also decreased macrophage infiltration, together with significant declines in chemotactic proteins including monocyte chemoattractant protein-1 (MCP-1) and MIP-1α. GW501516 also alleviated medial smooth muscle cell apoptosis and focal destruction of elastin [71]. These protective effects of GW501516 against aneurysm were correlated with enhanced expression of types I and III collagen, fibronectin, elastin and TGF-β1 [71]. PPAR-δ agonist also enhanced the expression of tissue inhibitor of metalloproteinase TIMP-2 and TIMP-3, while it strongly blocked MMP-2 expression [71]. These findings are consistent with previous studies with EA, also a ligand of PPARδ that effectively inhibits elastase and thrombin activity.

Schnegg et al. investigated if the PPARδ agonist, GW0742, could prevent radiation-induced cerebral injury and inflammation in C57Bl/6 wild-type (WT) and PPARδ knockout (KO) mice. Dietary GW0742 hindered the acute increase in IL-1β mRNA and ERK phosphorylation at 3 h after a single 10 Gy dose of whole body irrradiation (WBI); it also prevented the increase in activated hippocampal microglia one week after WBI [54].

PPARδ also regulates signaling pathways in human blood macrophages. A large fraction of direct PPARδ target genes are correlated with nonmetabolic functions, particularly with immunity, such as *CD300A*, *CD52*, *LRP5*, *NLRC4* and *PHACTR1*, and most of these are dependent on the type of agonists and target cell [55]. For instance, disruption of the *Cd300a* gene resulted in inflammatory activation of peritoneal macrophages in mice [55]. A prominent percentage of inflammation-associated NFκB and STAT1 target genes are repressed by PPARδ agonists [55]. First, PPARδ activation could downregulate several inflammatory cytokines (e.g. IL8, IFNG) and chemokines (e.g. CCL3/MIP1A, CCL8/MCP2, CCL11/eotaxin, CCL13/MCP4) [55]. Further, RNA-Seq analyses determined various essential components of NALP inflammasomes as new PPARδ targets [55].

16. Mitochondrial pathology and oxidative stress in ALD and anti-oxidative activity of erucic acid

A clinical trial with antioxidant agents in ALD is ongoing in Spain, since antioxidants reduce oxidative stress and spinal axonal injury in the Abcd1-knockout mice [56]. Human adrenal cortex and brain from ALD patients (juvenile and adult cerebral ALD and adrenomyeloneuropathy) demonstrate markers of oxidative injury, particularly lipid peroxidation (4-hydroxynonenal and malondialdehyde)-immunoreactivity in astrocytes and microglia [56]. Importantly, these changes in oxidative biomarkers were also found beyond the demyelinating limit [56]. In childhood ALD brain white matter, an increase in reactive lipid aldehydes (4-hydroxynonenal and acrolein) was also reported [56]. Enhanced levels of carbonyl proteins in all brain regions was detected in childhood ALD. Furthermore, reduction in plasma thiols was detected in childhood ALD, adrenomyeloneuropathy and Addison-only patients and higher plasma carbonyls were found in adrenomyeloneuropathy and childhood ALD patients compared with controls [56]. A significant negative correlation between thiols content and C26:0 was observed in the plasma of ALD patients, which indicate a link between C26:0 accumulation and protein oxidative damage in development of ALD [56]. VLCFAs can interfere with oxidative phosphorylation (OXPHOS) proteins and mitochondrial respiration, increasing ROS production [57]. Oxidative damage to OXPHOS subunits, Krebs cycle proteins, or to the pore component cyclophilin D induces a bioenergetic failure, leading to decreased ATP, NADH, and blockage of pyruvate kinase [57]. These abnormalities occur in relation to decreased mitochondrial biogenesis and proteasome dysfunction, preceding axonal degeneration in the Abcd1 knockout mice [57]. This axonal damage can be alleviated with an antioxidant cocktail. Furthermore, pioglitazone a PPARγ-agonist reduces ALD symptoms in the knockout mouse model in accompaniment with enhanced mitochondrial biogenesis. PPARγ is not a specific inducer of mitochondrial biogenesis but it may indirectly induce this process via enhancing PPAR co-activator PGC-1α [32].

A human study in 1996 showed that a rapeseed diet with low EA induces lesser malondialdehyde DNA adduct formation in comparison to soy bean diet [58]. Further, in a liposome oxidation model in vitro, EA (60 μg/ml) was found to act as a direct and potent antioxidant [59]. On the other hand, Lorenzo's oil treatment did not alleviate accumulation of blood thiobarbituric acid reactive species (TBARS) (markers of oxidative stress) in ALD patients [72], but these findings do not exclude the possibility that the intracellular EA would alleviate oxidative damage within the cytoplasm and individual organelle components. De Craemer et al. investigated peroxisomal alterations in mice treated with different doses of Lorenzo's Oil for up to 100 days. Hepatic EA levels were significantly increased 2.2-fold and 2.6-fold in mice treated with 10% and 20% Lorenzo's Oil for 21 days, respectively. Noteworthy, no lipidosis was found in liver, heart and kidney of any of the treated mice [60]. While hepatic and renal catalase activities were not induced by either diet, myocardial catalase activity was increased in most groups [60]. Catalase is a peroxisomal enzyme very important in protecting the cells against reactive oxygen species via catalyzing the decomposition of hydrogen peroxide to water and oxygen. Catalase has one of the highest turnover of all enzymes; one catalase molecule can convert

millions of hydrogen peroxide molecules to water and oxygen each second. Moreover, EA may also protect mitochondria and stimulate mitochondrial biogenesis, since it is a ligand of PPARδ [35].

17. Erucic acid and erucic acid-rich oils: nootropic effects

Kameyama et al. compared the behavior of mice fed with six different vegetable oils - corn, rapeseed, soybean, safflower, perilla and a mixture of perilla and safflower oils for 8 months after weaning [61]. In comparison to other oils, the rapeseed oil (an oil with high levels of EA) group exerted much higher locomotor activity, higher ambulation and rearing activities, slower habituation behavior and faster learning (measured by water maze task) as compared to controls [61]. Further, the differences in the alpha-linolenate/linoleate ratios of these oils did not account for the observed differences in the behavioral features among the six dietary groups [61]. In 2012, Arsenault et al. fed mice with diets either based on canola:soybean oils (40:10, g/kg) or safflower:corn oils (25:25, g/kg) to analyze the association between the lipid profile of brain fatty acids and the features of enthorinal cortex neurons [62]. Canola oil is derived from the EA-rich rapeseed oil; its EA content while reduced,still remains at upto 2%, while other edible oils consumed in the Western World almost lack any EA content. Canola:-soybean oil diet increased oleic acid and decreased arachidonic acid in ethanolamine glycerophospholipids of the white matter [62].

Mustard oils are rich in EA, and Indian mustard *Brassia juncea* is particularly rich in EA [63]. In 2013, Thakur et al. analyzed potency of a *Brassia juncea* extract for treatment of cognitive problems associated with diabetes or induced by central cholinergic dysfunction [64]. Elevated plus-maze and active- and passive-avoidance tests were employed to investigate the anti-amnesic potentials of *Brassica juncea* in alloxan-diabetic or scopolamine-treated rats [64]. Anti-amnesic effects of *Brassica juncea* in scopolamine-amnesia was not dose-dependent; but the same effect increased with döşe in diabetic rats. *Brassica juncea* dose dependently decreased the excessive amounts of acetylcholine esterase, and significantly enhanced the superoxide dismutase and catalase levels in brains of scopolamine-challenged and diabetic rats [64]. Oleic acid was previously shown to enhance the long-term potentiation (LTP) in the intact hippocampus via activating PKC [65]. Hence, EA, by being a precursor of oleic acid may also stimulate LTP; but by itself may also have nootropic effects. In 2016, it was investigated whether pure EA enhanced cognition or alleviated scopolamine-induced memory impairment, partly because the chemical structure of EA is very similar to that of oleic acid [65]. EA (3 mg/kg, p.o.) increased memory performance in normal naïve mice and alleviated scopolamine-induced memory impairment, as assessed by behavioral tasks [65]. The administration of EA increased the phosphorylation levels of phosphatidylinositide 3-kinase (PI3K), protein kinase C zeta (PKCζ), extracellular signal-regulated kinase (ERK), cAMP response element-binding protein (CREB) and additional protein kinase B (Akt) in the hippocampus [65]. PKC and particularly its subunit PKCζ increase LTP, which enhances persistence of synapses in the hippocampus. ERK is a downstream signaling molecule of PKCζ, and PKCζ can be activated through several kinases, including PI3K. PI3K also plays major roles in synaptic plasticity, the consolidation of recognition memory and hippocampal LTP [65]. Akt, another downstream molecule of PI3K pathway, phosphorylates different substrates and regulate multiple cellular processes including neuronal cell proliferation and synapse formation [65].

18. Erucic acid: concerns about cardiac toxicity

The natural docosenoic acid in the human diet can be predominantly EA, 22:1 ω9 or 22:1 ω11 depedning on whether it was obtained from plant source (generally rapeseed oil), or marine source, respectively [67]. Marine oils, however, also contain EA although in smaller amounts in comparison to the 22:1 ω11 [67]. Cetoleic acid 22:1 ω11, an isomer of EA, is highly present in Eskimo diet and it has a similar toxicity to EA in lower animals, but no toxicity is ascribed to cetoleic acid in Eskimo's [66,67]. Ackman et al. investigated fatty acids and plasma lipids of some Greenland Eskimos and they showed that the dietary docosenoic acid isomer distribution pattern for 22:1 ω11 was about 5 times > 22:1 ω9, a ratio typical of marine mammal fats [67]. A similar ratio was also found in the plasma fatty acids, but with a slightly higher proportion of 22:1ω9. The authors have concluded that when correlated with the excellent cardiovascular health of Greenland Eskimos, the current concerns regarding the detrimental impact of dietary docosenoic acids on the human myocardium, largely based on rat studies, may be misleading? [67]. They also highlight that a primate study with *Macaca fascicularis* for six months failed to show myocardial lesions from either EA or 22:1ω11 (the latter was modified by partial dehydrogenation) at 20 to 25% of the fat in a diet incorporating 25% fat [67].

Laryea et al. determined that, in Shaanxi Province of China, the main source of fat is rapeseed oil contributing about 44% of the total fat intake and 3% of the daily nutrient energy is provided by EA [73]. These authors have found prominent levels of EA in blood plasma and erythrocyte lipids in healthy children of this province [73]. While they did not describe any cardiac disease in their study population, they claimed that this region geographically lies in the Keshan area, where Keshan's Disease (selenium deficiency- associated digestive cardiomyopathy) was first described and therefore, proposed that Keshan's disease occurrence might be correlated to high EA and low selenium levels [73]. In our opinion, low doses of EA alone are not cardiotoxic and may be even protective; while selenium deficiency may cause a micromilieu, which might perturb the effects of EA. Alternatively or more likely additionally, simultaneous effects of other fatty acids (such as those in marine oils) also interfere with the risks and benefits of EA-consumption. No signs of lipidosis or cardiac fibrosis were found in human autopsies in India where consumption of mustard oil (with intermediate EA levels) was about 5.6% of the total fatty acid of the myocardial lipid [73].

19. Conclusions

The primary progressive subtype of MS occurs in approximately 10–20% of patients; and hitherto, no drug is approved by FDA to treat the primary progressive form. In a similar manner, no drug exists in AD to prevent disease progression and neuronal loss in the long- term. Hence, discovery of novel agents for both diseases is an urgent priority. Based on the studies highlighted in this review, EA should be tested in animal models to investigate whether it can exert neuro-protective and remyelinating functions. Successful completion of such studies would underscore its therapeutic potential and it can be further investigated in highly progressive and treatment refractory forms of MS and AD.

Conflict of interest

None.

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