Title: Possible role of Epoxyeicosatrienoic acid in prevention of oxidative stress mediated Neuroinflammation in Parkinson disorders

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To
The Editor
Medical Hypotheses

Respected Sir,

Sub.: Submission of research article for publication

Please find attached our research article entitled “Possible role of Epoxyeicosatrienoic acid in prevention of oxidative stress mediated Neuroinflammation in Parkinson disorders” for publication in the journal of Medical Hypothesis. I hereby declare that this article has not been published previously and all the authors involved in this work have given their approval for publication of this work in this journal. I also declare that, if accepted, this work will not be published elsewhere including electronically in the same form.

Kindly do the needful

Thanking you

Yours Sincerely

(Praveen T.K.)

From
Dr. Praveen T.K.
Assistant Professor
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To
The Editor
Medical Hypotheses

Respected Sir,

Sub.:--Submission of revised research article "Possible role of Epoxyeicosatrienoic acid in prevention of oxidative stress mediated Neuroinflammation in Parkinson disorders" for publication-Reg.

We have carefully considered the minor points raised by the reviewers and revised the manuscripts accordingly as per the following points raised:

1. Data has been provided that EETs or soluble epoxide hydrolase inhibition in the brain can increase blood pressure (Sellers KW et al. FASEB J 19:626-628, 2005). The findings in this publication need to be addressed.
2. Two other important references should be cited and their work discussed. The distribution of soluble epoxide hydrolase in the human brain has been determined (Sura et al. J Histochem Cytochem 56:551-559, 2008). Another study has evaluated behavioral phenotypes in traumatic brain injury in soluble epoxide hydrolase KO mice (Strauss et al. Prostaglandins and Other Lipid Mediators 104-105: 18-24, 2013).

Kindly do the needful

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From,
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Possible role of Epoxyeicosatrienoic acid in prevention of oxidative stress mediated Neuroinflammation in Parkinson disorders

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Keyword: sEH, EETs, PD, neuroprotection, Cytochrome P450, neuroinflammation, oxidative stress, apoptosis.

Abbreviations:  sEH, soluble Epoxide Hydrolase; mEH, microsomal Epoxide Hydroxylases; EET, Epoxyeicosatrienoic acids, PD, Parkinson’s disease; SNCA, Synuclein alpha; LRRK2, Leucine-rich repeat kinase 2; PARK2, E3 Ubiquitin-protein ligase parkin; PINK1, PTEN induced putative kinase 1; DJ-1, PARK7 - Protein deglycase; ATP13A2, ATPase Type 13A2; COX, Cyclooxygenase; LOX, Lipoxygenase; CYP 450, Cytochrome P450; PLA2, Phospholipase A2; DiHETEs, Dihydroxyeicosatrienoic acids; ROS, Reactive oxygen species; AUDA, (12-(3-adamantan-1-yl-ureido)-dodecanoic acid; GC-MS, Gas chromatography–mass spectrometry; LC-MS, Liquid chromatography–mass spectrometry; HPLC, High-performance liquid chromatography; MCA, middle cerebral artery; PPAR, Peroxisome Proliferator-activated receptor; TRPV4, Transient receptor potential cation channel; mitoKATP, Mitochondrial ATP-sensitive K+ channels; MAPK, Mitogen-activated protein kinase; PI3K, Phosphatidylinositol 3kinase; EDHF, Endothelium-derived hyperpolarizing factor; SN, Substantia nigra; ATO, Arsenic Trioxide; IL, Interleukins; TNF, Tumor necrosis factor; NF-κB, Nuclear factor κB; IKK, κB kinase; STAT3, Signal transducer and activator
of transcription 3; V-CAM, Vascular cell adhesion molecule; I-CAM, Intercellular adhesion molecule; TRPV1, Transient receptor potential vanilloid type 1; CNS, Central nervous system;

Abstract

Parkinson’s disease (PD) is a multifactorial neurodegenerative disease involving oxidative stress, neuroinflammation and apoptosis. Epoxyeicosatrienoic acids (EETs) are arachidonic acid metabolites and they play a role in cytoprotection by modulating various cell signaling pathways. This cytoprotective role of EETs are well established in cerebral stroke, cardiac failure, and hypertension, and it is due to their ability to attenuate oxidative stress, endoplasmic reticulum stress, inflammation, caspase activation and apoptosis. The actions of EETs in brain closely parallel the effects which is observed in the peripheral tissues. Since many of these effects could potentially contribute to neuroprotection, EETs are, therefore, one of the potential therapeutic candidates in PD. Therefore, by increasing the half life of endogenous EETs in vivo via inhibition of sEH, its metabolizing enzyme can, therefore, constitutes an important therapeutic strategy in PD.

Introduction

Parkinson’s disease (PD) is the second most common progressive neurodegenerative disorder with a characteristic symptoms such as bradykinesia, rigidity, resting tremor and posture instability (1-4). It is a multifactorial disease involving age, genetic and environment factors. Ageing is associated with mitochondrial dysfunction, increased free radical production and oxidative stress, which may lead to genomic instability and DNA mutations, with reduced survival (5-7). For the past 15 years, genetic characterisation of PD has shown sequence or copy number variants in at least six [(synuclein alpha (SNCA), leucine-rich repeat kinase 2 (LRRK2), E3 ubiquitin-protein ligase parkin (PARK2), PTEN induced putative kinase 1 (PINK1), PARK7 - Protein deglycase DJ-1, ATPase Type 13A2 (ATP13A2)] which have
been identified to cause monogenic forms of PD (7, 8). Environmental factors such as head trauma and exposure to pesticides (rotenone, paraquat, dieldrin, etc.), solvents (trichloroethylene, carbon tetrachloride, n-hexane etc.), and metals (lead, iron, manganese etc.) are reported to cause destruction of dopaminergic neurons through oxidative and inflammatory reactions (9). The morphologic hallmark of PD is the presence of alpha-synuclein (α-syn)-rich Lewy bodies within the dopaminergic neurons, which are mainly formed due to mutations in α-syn gene, leading to protein aggregation. Lewy body formation is observed both in familial and sporadic forms of PD (10-14). Molecular level analysis of PD confirm that oxidative stress, mitochondrial dysfunction and neuroinflammation are the major contributing factors (15).

Plasma membrane arachidonic acid (AA) released by phospholipids by phospholipase A2 (PLA2) is metabolized to prostaglandins and thromboxane by cyclooxygenase (COX), to leukotriens by lipoxygenase (LOX), and to epoxyeicosatrienoic acid (EETs) by cytochrome P450 (CYP450) oxidases (Figure 1) (16, 17). Four EETs regioisomers, 5,6-, 8,9-, 11,12-, and 14,15-EET are produced by CYP450 epoxygenases pathway. These regioisomers are quickly metabolized to inactive or less active metabolites by soluble or microsomal epoxide hydroxylases (sEH or mEH) and their estimated *in vivo* half-life is of few seconds to minutes (18) (Figure 1 and 2). EETs are present in heart, lungs, kidneys, gastro-intestinal tract and in brain (19, 20). Sura et al., reported the preferential expression sEH in neuronal cell bodies, oligodendrocytes, astrocytes, meningeal blood vessels, and in choroid plexus of human brain (20). EET’s are reported to exhibit anti-inflammatory and antioxidant properties which protect against mitochondrial dysfunction and apoptosis and play an important role in regulation of cerebral blood flow (21). A diverse class of agents such as amides, ureas, thioamides, thioureas, carbamates, acyl hydrazones, chalcone oxdies, etc., has been reported to possess sEH inhibitory potential (22). Some of the sEH inhibitors have been extensively
studied for their cytoprotective benefits in hypertension, ischemic heart disease, heart failure, renocardiac failure, diabetic neuropathy, cancer and obesity (23-31). Although EETs are widely distributed in the brain, not much research has been carried out to exploit their cytoprotective benefits in the treatment/prevention of PD (25, 32).

**The hypothesis proposed**

The cytoprotective properties of EETs have been well established in various peripheral disorders and they may play a similar role in the brain cells. The cytoprotective effect of EETs, however, is limited by their metabolism via soluble epoxide hydrolase (23, 25, 33-38). Therefore, we hypothesize that increasing the half life of endogenous EET’s through inhibition of its major metabolizing enzyme, soluble epoxide hydrolase (39), is, therefore a novel approach to prevent/treat the PD (21, 40-44).

**Justification of proposed hypothesis**

The pathogenesis of PD is associated with oxidative stress, mitochondrial dysfunction, protein aggregation, misfolding, inflammation, excitotoxicity, and apoptosis (45). Oxidative stress results due to overproduction of reactive species or a failure of cell buffering mechanisms that normally limit their accumulation. This oxidative stress results in damage to proteins, lipids, and nucleic acids has been found in the substantia nigra (SN) of PD patients (46). The ROS production occurs due to a variety of factors including dopamine metabolism, exposure to environmental toxins, mitochondrial dysfunction, probably all of which can result in inhibition of mitochondrial Complex I activity in the SN of PD patients (45-47). PD patients display impairment of endogenous protective mechanism such as lowered antioxidants such as glutathione, superoxide dismutase, etc. EETs have been reported to
promote endogenous mechanisms to buffer free radicals, thereby reducing the oxidative damage to sub-cellular organelles (21, 48). A study by Liu et al (76) demonstrated that EETs attenuate oxidative stress, mitochondrial dysfunction, caspase activation, and apoptosis in carcinoma cells treated with arsenic trioxide (ATO) (75). The results showed that pretreatment with 11,12-EET increased the expression of the antioxidant enzymes superoxide dismutase and catalase and inhibited ATO-induced apoptosis and activation p38 mitogen-activated protein kinase, c-Jun NH2-terminal kinase, caspase-3, and caspase-9, which could have potential neuroprotective and therapeutic implications for PD (49). However the specific signalling mechanisms by which EETs exert their direct protective effects in astrocytes and neurons still remain unclear.

Neuroinflammation secondary to oxidative stress is one of the primary mechanisms involved in PD. Elevated pro-inflammatory cytokines, such as IL-1β, IL-6, and TNF-α have been reported in the SN of PD patients (50). The accumulation of alpha-synuclein (α-syn)-rich Lewy bodies has been reported to directly trigger a microglial response and release of cytotoxic factors (51). Anamitra Ghosh and co-workers have reported that selective inhibition of NF-κB prevents dopaminergic neuronal loss in a mouse model of PD. The study evaluated the ability of a peptide corresponding to the NF-κB essential modifier-binding domain (NBD) of IkB kinase (IKK) or IKKβ to prevent nigrostriatal degeneration in the MPTP mouse model of PD (52). The NBD peptide reduced nigral activation of NF-κB, which in turn suppressed the nigral microglial activation, protected both the nigrostriatal axis and neurotransmitters, and improved motor functions in the MPTP model (52). The anti-inflammatory actions of EETs have also been attributed to cytokine-activated nuclear factor-κB (NF-κB) -mediated transcription. This in turn results in inhibition of IKK phosphorylation of IkBa, resulting in
reduction in the plasma levels of pro-inflammatory cytokines and nitric oxide metabolites (38, 53).

In addition to NF-κB inhibition, the EETs are also reported to activate STAT3 receptor, thereby promoting STAT3 tyrosine-705 phosphorylation and nuclear translocation which plays an important role in IL-10-mediated anti-inflammatory signaling and gene expression (54). EETs are also reported to reduce the expression of the leukocyte adhesion proteins such as vascular cell adhesion molecule (V-CAM), intercellular adhesion molecule (I-CAM) and E-selectin (37), thereby reducing the number of leukocytes induced activation of microglial cells mediated inflammatory damage.

The anti-inflammatory effects of EETs could also be caused via transient receptor potential vanilloid type 1 (TRPV1), which is activated in brain due to heat, endogenous lipid molecules and oxidative stimuli (55) and also due to exogenous agonists such as evodiamine (an active ingredient of the evodia fruit) and capsaicin (an active ingredient of hot pepper). Activation of TRPV1 permits calcium (Ca\(^{2+}\)) entry, which results in an elevated level of intracellular Ca\(^{2+}\) which serves as a signal to elicit anti-inflammatory responses in neurons (56, 57). In addition to anti-inflammation, EETs are reported to activate PPAR-γ thereby promoting various physiological processes such as fatty acid and glucose metabolism, angiogenesis, cellular proliferation and differentiation, (58). The activation of PPAR-γ are reported to suppresses the NF-κB mediated expression of molecules such as VCAM-1, ICAM-1, and endothelins that are involved in the inflammatory response (56). They also increase the endogenous antioxidant levels of glutathione, SOD, catalase etc. There are several studies which have reported the neuroprotective properties of PPAR-γ agonists in PD (59).
The dopaminergic neurons in the SN of PD patients have reported increased glutamate
receptors, and receive glutamatergic innervations from the subthalamic nucleus and cortex.
The excessive NMDA receptor activation by glutamate increases intracellular Ca\textsuperscript{2+} levels
which in turn activate cell death pathway (60). The intracellular Ca\textsuperscript{2+} is sequestered regularly
into the endoplasmic reticulum and mitochondria to prevent the activation of cell death
pathways. Due to increased oxidative stress, mitochondrial dysfunction there is imbalance in
Ca\textsuperscript{2+} levels (61). The Blockade of L-type Cav1.3 calcium channels are reported to decreases
the severity of PD. The excitotoxicity of glutamate is reported to be mediated by sustained
increase in the cytosolic Ca\textsuperscript{2+} concentration. EETs have been reported to inhibit cardiac L-
type calcium channels which play an important role in regulating cardiac contractility, heart
rate etc (62). Similar mechanisms, may sequester the excessive Ca\textsuperscript{2+} overload and Ca\textsuperscript{2+}
mediated glutamate excitotoxicity in PD (25, 37). Isradipine a L-type calcium channel
inhibitor is in the Phase III clinical trial, which has therapeutic potential in slowing the
progression of the PD in pre-clinical studies (63). The role of EETs in the brain and CNS
appears to closely parallel the functions as described in other peripheral tissues (21).

Apart from the above proposed mechanisms, the signalling of by EETs is involved in the
process that is distinct to CNS functions. EETs modulate neuronal pain processing in the
brainstem. The CYP oxidase metabolic pathway interacts with the neuroactive
endocannabinoid pathway (48), which plays an important role in regulating neurohormone
release from neuroendocrine regions of the brain. The function of EETs in the neurogenic
regulation of cerebral blood flow suggests that EETs may be key regulators of synaptic
transmission, a function which is distinct to CNS (37). Strauss et al., evaluated the effect of
traumatic brain injury (TBI) on behavioural phenotypes in soluble Epoxide Hydrolase
Knockout Mice (Ephx2-KO). They report that the Ephx2-KO mice showed improved motor
coordination in beam walk test when compared to wild-type and a minor impairment in
working spatial memory independent of TBI in Morris water maze test. The results of this study show that sEH deficiency interacted both with neurologic and cognitive performance, independent of brain injury (64).

Experimental works carried out by Xiaocui Qin et al., on MPTP model of mice and primary cortical neuronal cell cultures, have reported the increased expression of sEH in MPTP-treated mice. The study reported sEH deficiency and inhibition significantly attenuated tyrosine hydroxylase (TH)-positive cell loss and improved rotarod performance in mice. Data suggested that sEH inhibition might be a powerful tool to protect dopaminergic neurons in PD (65). Terashvili et al., performed mechanistic studies on co-cultures of astrocytes and N27 dopaminergic neuron and reported that EETs (released from astrocytes and neurons) enhanced cell viability against ROS induced injury. Further, sEH inhibitor (12-(3-adamantan-1-yl-ureido)-dodecanoic acid (AUDA) increase the EET levels, thereby increasing the neuronal viability and projecting the cytoprotective ability of EETs against hydrogen peroxide induced oxidative stress in brain (40).

One of the drawbacks associated with EETs is their pro-mitogenic action which may result in uncontrolled cell division and cancer. However, Emun Abdu et al., have reported the administration of regioisomeric mixture of EETs resulted in a concentration dependent increase in axon outgrowth in primary sensory and cortical neuronal cell cultures. This suggested a novel therapeutic use of EET’s in promoting nerve regeneration (66). Munzenmaier et al., have proved that the promitogenic actions of EETs resulted in the formation of endothelium tube, when the cerebral microvascular endothelial cells were cocultured with astrocytes (67). EETs have also been reported to play an important role in promoting angiogenesis in cerebral vasculature which is due to its promitogenic potential (21,
36, 48, 68). Thus the promitogenic property of EET exhibit positive or negative actions on CNS is still under research.

In peripheral vasculature, sEH inhibitors are reported to exert vasodilatory effects (24). However, in the cardiovascular regions of brain such as brainstem they are reported to increase blood pressure (BP) and heart rate (HR) in spontaneously hypertensive rats but not in Wistar Kyoto rats (69). Hence the central role of sEH inhibitors in hypertension is not yet clear.

**Conclusion**

Since the pathogenesis in PD is multifactorial, novel compound(s) that simultaneously target multiple degenerate pathways are required. In this regard, our hypothesis on the potential therapeutic effects of EETs is viable since EETs protect against oxidative stress, mitochondrial dysfunction, neuroinflammation and apoptosis. Administration of sEH inhibitors could be tested in various PD models, such as LPS, MPTP, and rotenone. Further, since EETs are naturally occurring endogenous compounds, their elevation might not pose neurotoxicity or systemic toxicity. However, additional experiments are required to understand the solubility of sEH inhibitors, their ability to cross the BBB and bioavailability in the brain. However, it needs to be seen whether such approaches could be tested as independent or adjunct therapy along with existing drug therapy with dopamine replacement.

**Conflict of interest:**

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Disclosures

The author declares that there is no conflict of interest.

References


Figure 1: Arachidonic acid pathway in astrocytes, dopaminergic neurons, and cerebral blood vessels. AA - arachidonic acid, COX-2 - cyclooxygenase-2, LOX - lipoxygenase, EET’s - epoxyeicosatrienoic acids, sEH - soluble epoxide hydrolase, CYP2C - cytochrome p450 2C, CYP2J - cytochrome p450 2J, DHETs - dihydroxyeicosatrienoic acids, cPLA - phospholipase A.
Figure 2: Intracellular signaling pathways of EET’s

Epoxideicosatrienoic acids exhibit both autocrine and paracrine signalling mechanism, and they are either directly released from the phospholipids stores or synthesized from the arachidonic acids (AA) via a cytochrome P450 pathway. The actions of EET’s are mediated by various intracellular signalling pathways as shown in blue colour bubbles, which get activated at specific conditions in different tissues. The activation of large-conductance Ca-activated K (BKCa) channels occurs through a Gs protein coupled to the putative receptor or due to activation of transient receptor potential vanilloid type 1. The cAMP-PKA, phosphatidylinositol 3-kinase (PI3K)-Akt, mitogen activated protein kinase (MAPK) pathways produce response by activation of gene expression. Src and tyrosine kinase promote phosphorylation of specific proteins and promote various intracellular signalings such as mitogenesis. The activation of PPRAαγ inhibits the NF-kappa B action via IKK, IκBα and also reduce the expression of the leukocyte adhesion proteins such as V-CAM, I-CAM and E-selectin. STAT3 tyrosine promotes phosphorylation and nuclear translocation which inhibits IL-10-mediated inflammatory signaling and gene expression. Heparin-binding EGF-like growth factor (HB-EGF) is the protein encoded by HBEGF gene. AA- arachidonic acid, EET’- epoxideicosatrienoic acids, sEH - soluble epoxide hydrolase, CYP2C - cytochrome p450 2C, CYP2J - cytochrome p450 2J, DHETs - dihydroxyicosatrienoic acids, cPLA - phospholipase A
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