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Inhibitors of soluble epoxide hydrolase and cGAS/STING repair defects in amyloid-β clearance underlying vascular complications of Alzheimer's disease

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Abstract

Background: Alzheimer's disease (AD) and its monoclonal antibody therapies are associated with brain vasculitis and amyloid-related imaging abnormalities. The naturally-formed epoxides (EpFAs) of polyunsaturated fatty acids (PUFAs), such as 11,12-epoxyeicosatetraenoic acid (EEQ), are anti-inflammatory and pro-resolution mediators, which are increased by dietary supplementation with ω-3 PUFAs. EpFAs are, however, enzymatically hydrolyzed by soluble epoxide hydrolase (sEH) in AD patients' macrophages in vivo and in vitro.

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Author contributions

Milan Fiala (Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Validation; Writing – original draft); Bruce D Hammock (Conceptualization; Funding acquisition; Methodology; Resources); Sung Hee Hwang (Methodology; Resources); Julian Whitelegge (Investigation; Resources); Ketema Paul (Investigation; Resources); Karolina Elżbieta Kaczor-Urbanowicz (Data curation; Formal analysis); Andrzej Urbanowicz (Formal analysis); Santosh Kesari (Investigation; Resources).

Declaration of conflicting interests

Bruce D Hammock and Sung Hee Hwang in the Company Eicosis, Inc, Davis, CA are developing the EC5026 inhibitor of soluble epoxide hydrolase for therapy of Alzheimer's disease. The remaining authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Objective: To repair amyloid-β 1–42 (Aβ) degradation by AD macrophages using the inhibitors of a) soluble epoxide hydrolase (sEHIs), termed TPPU and EC5026, together with EpFAs, or b) STING pathway termed H-151.

Methods: Immunobiology, immunochemistry, RNA sequencing, and confocal microscopy were used.

Results: In AD brain (examined postmortem), monocyte/macrophages upload Aβ in plaques and transfer it without degradation into brain microvessels, suffer apoptotis, and release Aβ, inducing vasculitis. The EpFAs of epoxyeicosatetraenoic acid (EEQ), along with the inhibitors TPPU and H-151, decrease inflammatory cytokines and regulate macrophage unfolded protein response to endoplasmic reticulum stress. Treatment of AD macrophages by TPPU with EEQ or by STING inhibitor H-151 increased uploading of Aβ after 2 hours and increased degradation of Aβ after 24 hours.

Conclusions: The sEHI inhibitor EC5026 and the STING inhibitor H-151 increased macrophage uptake and degradation of Aβ. EC5026 administration was safe in normal volunteers. EC5026 together with ω-3 PUFA supplementation are indicated for in a clinical trial in patients with mild cognitive impairment.

Keywords

Alzheimer’s disease; amyloid-beta; EC5026; H-151; monocytes/macrophages; soluble epoxide hydrolase; TPPU; vascular complications

Introduction

Failure of amyloid-β (Aβ) degradation by macrophages of Alzheimer’s disease (AD) patients, especially in those treated by monoclonal antibodies, is acutely associated with myeloid vasculitis and, over time, with cerebral amyloid angiopathy (CAA).

According to the amyloid hypothesis, the deposition of Aβ in plaques, neurons, and blood vessels is the proximal cause of AD.[1] AD brain contains oligomeric and soluble Aβ in neurons and apoptotic macrophages in perivascular spaces, and insoluble Aβ and fibrillar P-tau in plaques and blood vessels.[2] Novel therapies by the monoclonal Aβ antibodies aducanumab and lecanemab may, although controversially, slow AD progression[3–5] and increase Aβ clearance (demonstrated by brain imaging). Monocyte/macrophages (MMs) upload Aβ in plaques, transfer and release it into brain microvessels and disrupt the blood-brain barrier (BBB),[2] and, in some patients, induce myeloid vasculitis and CAA, brain edema, and hemorrhagic stroke and amyloid-related imaging abnormalities (ARIAs).[6] CAA also develops with aging alone. Thus, MMs in the AD brain are a two-edged sword: MMs upload and clear Aβ in the brain parenchyma but also disrupt the BBB and cause vasculitis by Aβ.[7]

Methods

Investigation of peripheral blood monocytes/macrophages

We examined the effects of the soluble epoxide hydrolase inhibitor (sEHI) TPPU together with epoxides of fatty acids (EpFAs) on the transcriptome and Aβ degradation of MMs generated from peripheral blood mononuclear cells (PBMC) of two mild cognitive impairment (MCI) patients (#1 and #2). The diagnostic evaluation was performed by Mini-Mental State Examination (MMSE). Patient #1, age 68, male gender; MMSE 21/30 points; *APOE* ϵ3/ϵ4 genotype; patient #2, age 71, male gender; MMSE 26/30 points, *APOE* ϵ3/ϵ3 genotype. This work was performed in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans and with the approval of the University of California, Los Angeles, by the Institutional Review Board. UCLA serves all ethnicities and genders.

Immunopathology of AD and control brain tissues

The postmortem brain of MCI patient #2 (patient #2 in Figure 1 in[8]) was stained at UCLA Department of Pathology by immunochemistry for Aβ ($A\beta^+$) and macrophages ($CD68^+$).

Chemicals

The methyl esters of EpFAs and the sEHI TPPU were prepared in the laboratory of B. Hammock, according to the previously described synthesis methods.[9] Lipophilic fatty acid epoxides and enzyme inhibitors were added to macrophages in dimethylsulfoxide (DMSO) (less than 1% final volume of cell culture medium) was used as a control.

Macrophage culture and treatments

We isolated PBMC from the blood samples of two AD patients by the Ficoll-Hypaque technique and cultured them in Iscove's Modified Dulbecco's Medium (IMDM) containing 10% autologous serum when typical CD68+ macrophages developed. In the experiments, we added the compounds as a DMSO solution at the final concentrations 1 μM EEQ or epoxydocosapentaenoic acid (EDP), 100 nM TPPU, or H151 at 100 ng/ml.

RNA sequencing and analysis

Total RNA was isolated from pure PBMC-derived macrophage cultures using Quick RNA Miniprep kit (Zymo Research, Inc.). Final libraries were generated from total RNA using KAPA mRNA Hyper kit, and quantified and sequenced on NovaSeq 6000 (Illumina, Inc). After removing low-quality reads, the data were aligned to the human genome GCHr38 using STAR (v2.6) and count data were normalized using DESQ2's median of ratios method. We compared the effects of EpFAs in normalized counts of macrophage samples and performed a supervised graphical analysis of individual genes of interest, according to the previous method.[8]

Results

Macrophages in AD brain upload, transfer, and release Aβ into vessels, causing vasculitis and CAA

The plaques and perivascular space in the brain tissues of AD patients,[2] including those of patients treated by monoclonal Aβ antibodies,[6] are infiltrated by CD68+ macrophages (Figure 1a,c), whereas normal or epileptic patients' brains are free of macrophages (Figure 1b). MMs are attracted into plaques by chemokines,[2] upload and clear Aβ in plaques (Figure 1c). The phagocytic cells in plaques are macrophages grossly distended with ingested Aβ (diameter 200 μM), whereas microglia (diameter < 20 μM) around plaques are free of Aβ (Figure 1c).

MMs transfer and release Aβ into brain vessels without degradation, producing heavy Aβ deposits at the vessel contact and lighter deposits indicative of CAA developing over time (Figure 2a,b).

Macrophages infiltrating AD brain are pro-inflammatory

The transcripts of AD patients' macrophages in comparison to caregivers' macrophages[8] are changed in: a) downregulation of the transcripts of energy and degradation enzymes: aa) OX-PHOS energy enzymes; bb) the degradation enzymes i.e., angiotensin-converting enzyme (ACE), membrane metalloendopeptidase, and insulin-degrading enzyme (IDE); cc) the enzymes in the ubiquitin-proteasome system E1, E2, and E3; b) upregulation of inflammatory cytokines[8] in cGAS/STING pathway. These changes in the macrophage transcriptome are associated with a pro-inflammatory phenotype, increased endoplasmic reticulum (ER) stress, and defective uptake and degradation of Aβ[2] and the pathology of AD brain.

Inflammatory signaling in macrophages is blocked by epoxides of fatty acids and the inhibitors EC5026 and H-151

Cytosolic foreign or autologous DNA activate cGMP-AMP synthase-STING (cGAS-STING) pathway in AD and other diseases.[10–12] cGAS signaling is important in neurological diseases (Parkinson's disease, AD,[13] amyotrophic lateral sclerosis), heart failure, pancreatitis, and cancer.

Non-degraded Aβ in macrophages causes inflammation through cGAMP-STING (cGAS) pathway, ER stress, and vasculitis.[14] cGAS is blocked by the STING inhibitor H-151.[10]

EpFAs are formed by cytochrome P-450 enzymes from polyunsaturated ω-3- and ω-6 fatty acids (PUFAs). EpFAs are lipid mediators that act in vivo in a homeostatic fashion by modulating the ER stress pathway to reduce inflammation and nociceptive pathophysiology.[15] ω-3 epoxides are more resistant to soluble epoxide hydrolase (sEH) hydrolysis than ω-6 epoxides and are poor substrates for inflammatory pathways leading to inflammatory prostaglandins. EpFAs[16] are, however, enzymatically hydrolyzed by sEH to more polar and usually inactive diols (Figure 3).[15] The sEHIs, TPPU and EC5026, effectively inhibit the degradation of EpFAs by sEH. In vivo, ω-3 fatty acid supplementation

increases the epoxides of arachidonic acid called EETs, the epoxides of ω-3 fatty acid eicosapentaenoic acid (EPA) called EEQs, and the epoxides of the ω-3 fatty acid DHA EDPs.

Thus, two approaches are potentially therapeutic by modulation of the inflammatory macrophage phenotype to an anti-inflammatory macrophage phenotype: a) inhibition of cGAS/STING by a STING inhibitor (e.g., H-151); b) blockade of sEH by the sEHI termed TPPU (dual inhibitor of sEH and acetylcholinesterase)[17] or the sEHI termed EC5026 (shown as safe in ongoing human studies[18]).

The objective of this study has been to inhibit inflammatory activation and improve macrophage clearance and degradation of Aβ in a safe fashion using two inhibitory drugs: EC5026[18] against sEH and H-151[10] against cGAS/STING pathway, before their use in a clinical trial. EC5026 has been tested in normal volunteers and found safe. In this study, the analogue of EC5026 termed TPPU was used instead.

Epoxides of EpFAs and sEHI regulate macrophage transcriptome in energy, Aβ degradation enzymes, and inflammatory cytokines in a homeostatic fashion

We analyzed in macrophages of two AD patients in vitro the effects of the sEHI termed TPPU with an epoxide of fatty acids on macrophage transcriptome. The sEH inhibitor TPPU and the EpFA termed EDP, regulated the transcripts of the Aβ-degrading enzyme ACE and insulin-degrading enzyme IDE, in a homeostatic anti-inflammatory fashion (Figure 4).

The EpFA EDP regulated the transcripts of granzyme B and inflammatory cytokines IL1B, IL-23A, IL-6, and TNF in a homeostatic fashion, i.e., downregulated highly-expressed transcripts and upregulated poorly-expressed transcripts (Figure 5).

Omega-3 epoxides increase the unfolded protein response to ER stress in comparison to omega-6 epoxides

The regioisomeric epoxide EDP upregulated XBP1, ATF4, and ATF6, which regulate unfolded protein response in folding enzymes, chaperones, and ER-associated degradation of denatured proteins (Figure 6).

Inhibitor of sEH termed TPPU and inhibitor of cGAMP/STING (cGAS) pathway termed H-151 increase Aβ in macrophages

Given the anti-inflammatory health benefits of PUFAs and EpFAs and PUFAs,[14] we speculated that sEHIs with EpFAs will be beneficial against vasculitis by protecting EpFAs against degradation by sEH.

Treatments by H-151 or EEQ with TPPU or H-151 were both active in comparison to DMSO a) to signficantly increase uptake of Aβ at 2 h ($p < 0.0000$) (in descending order) aa) H-151, bb) EEQ + TPPU versus cc) DMSO; b to significantly decrease residual Aβ load (in descending order) aa) EEQ + TPPU, or bb) H-151 versus cc) DMSO (Figures 7a,b).

Discussion

Movements to a disease-modifying therapy of AD: Successes and controversies

In an aging AD mouse model, the restoration of cellular bioenergetics through inhibition of the prostaglandin E_2 receptor 4 (EP4) signaling in myeloid cells restored cognition.[19] In human patients, a natural approach with PUFA supplementation delayed dementia, but it did not complete Aβ degradation and did not stop disease progression.[8,14] Several studies have indicated that sEHIs used alone offer a promise in the treatment of a number of chronic neurological diseases.[20–23] Following their discovery in the 1970s and early characterization, the first clinical targets of sEHIs were cardiovascular pathologies. sEHIs alone, presumably by increasing EpFAs, reduced vascular inflammation and atherosclerosis, thus protecting cerebral vasculature from AD-associated damage.

This study shows that the STING inhibitor H-151 and the sEHI inhibitor TPPU (analogue of EC5026) together with EpFAs have directly positive effects against AD through an early increase of Aβ uptake and a late increased degradation of Aβ. These in vitro studies of AD macrophages suggest that the inhibition of sEH in macrophages of AD patients could be clinically beneficial and should be tested in a future clinical trial. In addition, a combined inhibition by inhibitors of sEH and prostaglandin signaling should be examined as done in cancer therapy.[24]

EC5026, a compound similar to TPPU, has completed human safety trials without adverse effects on escalating oral doses from 1 mg per day.[25] The recommendations of the best AD therapy will be guided by clinical experience yet, these three agents (TPPU or EC5026, H-151, and ONO), offer a promise of reducing the adverse effects of monoclonal antibody therapies and directly improving AD therapy.

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Data availability

The data supporting the findings of this study are available within the article and figures.

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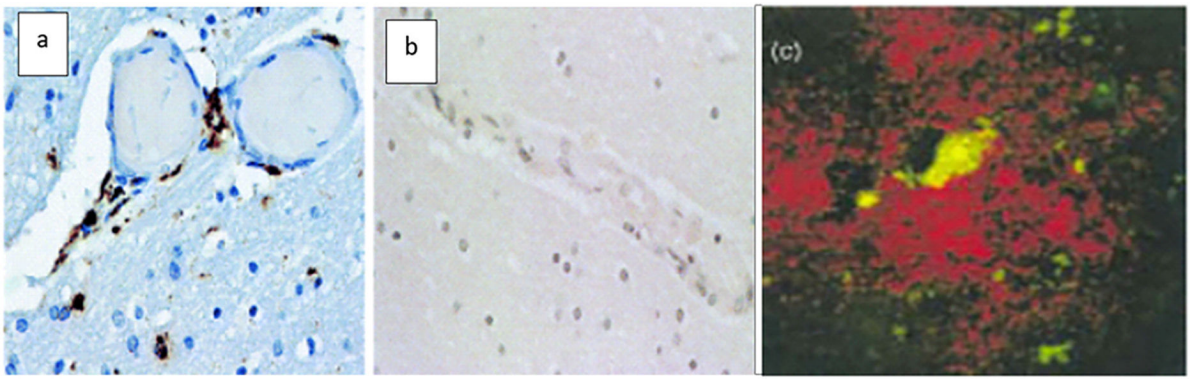

Figure 1.

Immunopathology of AD brain encompasses myelomonocytic vasculitis. Immunochemical study of the brain of the AD patient #2 (Dover et al., *J Alzheimers Dis* 91, 245–261. Copyright 2023, with permission from IOS Press) demonstrates: a) Perivascular macrophages (demonstrated by immunostaining as CD68-positive) in the AD brain #2; b) No perivascular macrophages in the brain of an epileptic patient; c) Macrophages upload amyloid-β (Aβ) in Aβ plaques (AD brain #2). Interpretation: CD68-positive cells in plaques are macrophages distended with Aβ (cell diameter ~200 micron), and CD68-positive outside of the plaque are 20 micron diameter CD68-positive, Aβ- negative microglia. Magnification a) and b) 20x; c) 100x.

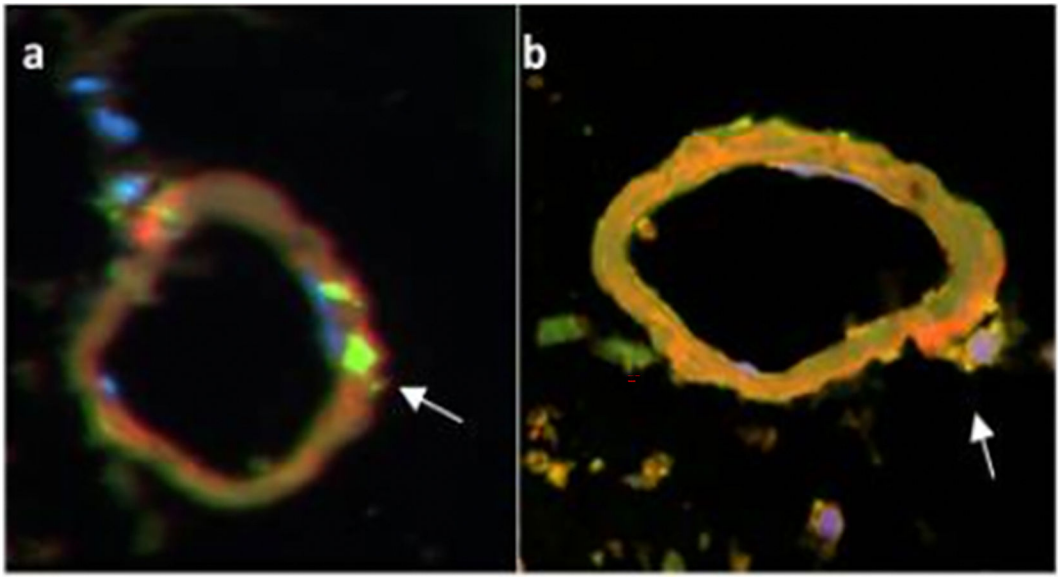

Figure 2.

Migration of monocyte/macrophages across the blood-brain barrier at brain microvessels causes congophilic amyloid angiopathy. Each CD68-positive macrophage abutting a brain/microvessel interface released heavy Aβ deposits underneath at a) 11 o'clock, b) 4 o'clock (arrow); and released Aβ in layers elsewhere (indicating CAA). Confocal microscopy of the brain of AD patient #2 (CD68 antibody (clone KP1, DAKO, Inc); Aβ antibody). Magnification 100x. (Dover et al., *J Alzheimers Dis* 91, 245–261. Copyright 2023, with permission from IOS Press).

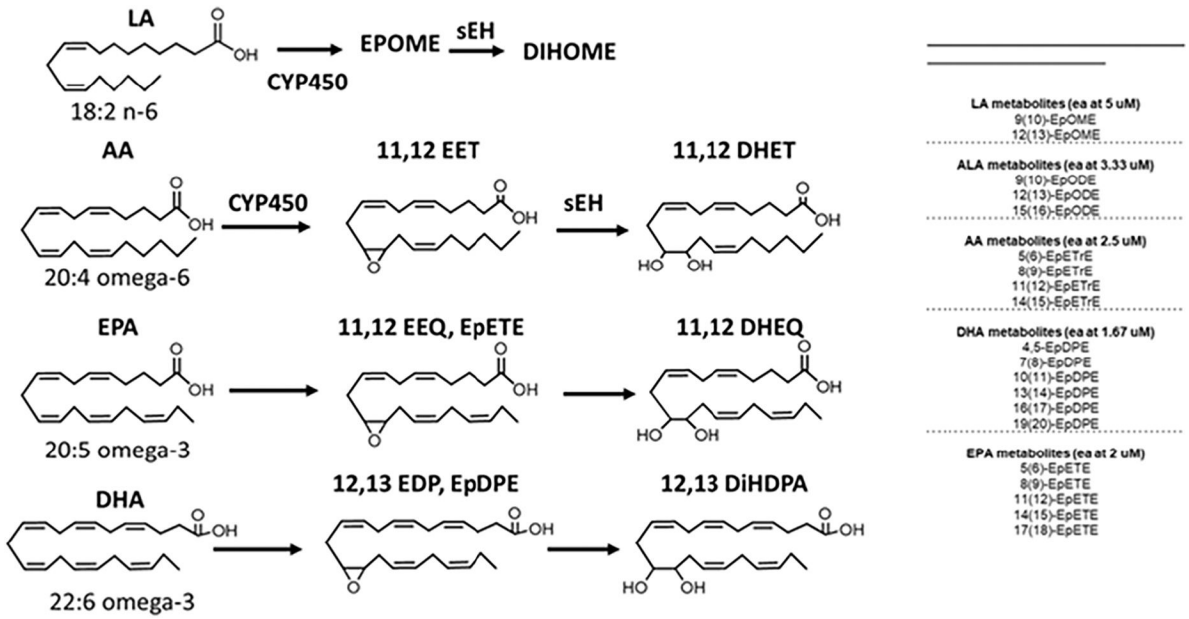

Figure 3.

Epoxy fatty acids (EpFAs). EPFAs are produced from polyunsaturated fatty acids (PUFAs) by cytochrome P450s and are degraded by sEH. EETs are produced from ω-6 arachidonic acid (AA), EEQ from ω-3 eicosapentaenoic acid (EPA), and EDPs from ω-3 docosahexaenoic acid (DHA). Only one regioisomer of EETs, EEQs, or EDPs is shown for clarity.

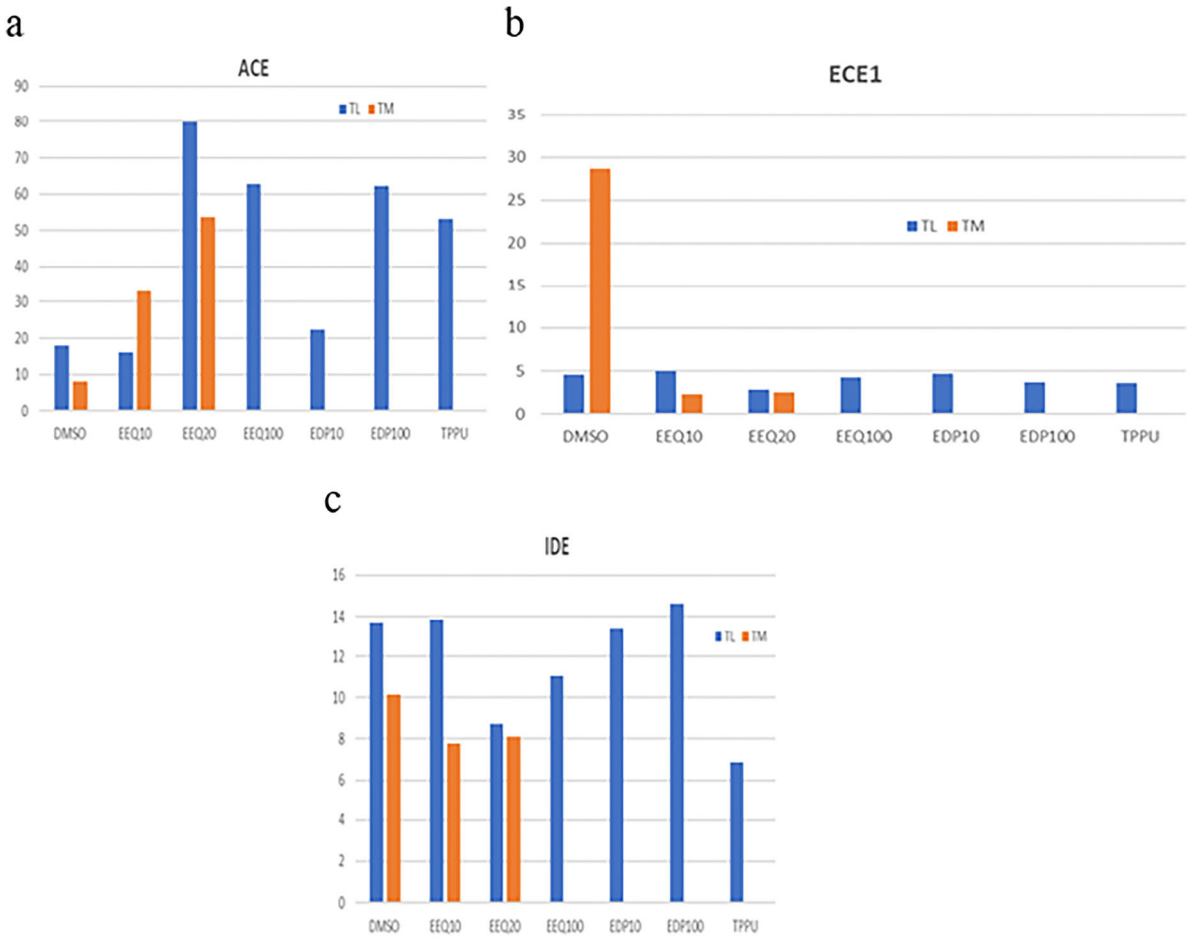

Figure 4.

Epoxides of fatty acids (EpFAs) regulate the transcripts ACE and IDE in AD patients' macrophages. In macrophages of two Alzheimer's disease patients: (a) EEQ and EDP upregulated Aβ-degrading enzyme angiotensin-converting enzyme (ACE), (b) EEQ downregulated the vasoconstrictor enzyme endothelin-converting enzyme (ECE1); (c) EpFA effects on insulin-degrading enzyme (IDE) were divergent in each patient. The vertical axis indicates transcripts (RPM) of each enzyme. When the bar is missing, no transcript was detected. EpFA and inhibitors were tested in macrophage cultures at the final concentrations 1 μM EEQ, 100 nM TPPU, or H151 at 100 ng/ml in DMSO.

Figure 5.

EpFAs regulate inflammatory cytokines of AD macrophages in a homeostatic fashion: In macrophages of two AD patients, highly-expressed transcripts were downregulated and lowly-expressed transcripts were upregulated. The vertical axis indicates the number of transcripts (RPM). EpFAs and inhibitors were added to macrophage cultures at the final concentrations 1 μM EEQ, 100 nM TPPU, or H151 at 100 ng/ml.

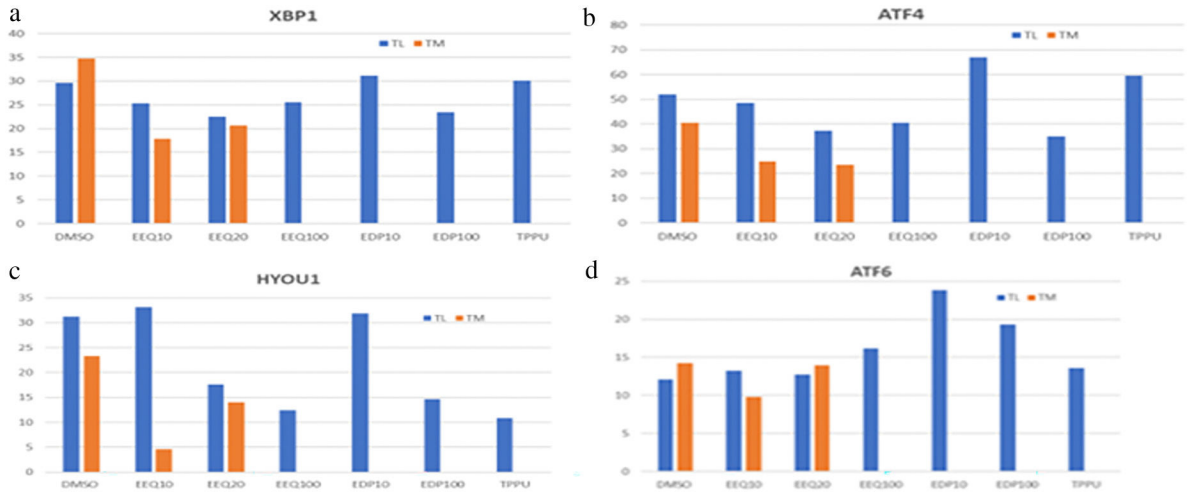

Figure 6.

The ω-3 epoxide EDP increased unfolded protein response (UPR) in transcripts of XBP1, ATF4, and ATF6 for immunity, apoptosis (C/EBP homologous protein (CHOP)), and chaperones. (a) EDP upregulates XBP1 for immunity; (b) EDP upregulates ATF4, which activates cell stress response in autophagy and CHOP; (d) EDP upregulates ATF6, which activates chaperones; (c) EDP effects on HYOU1 were minor.

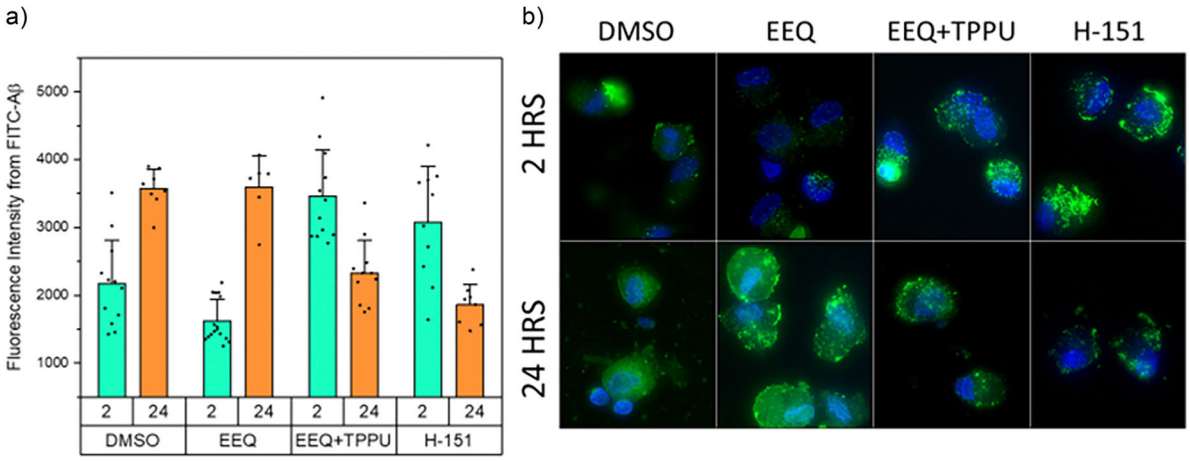

Figure 7.

EpFAs increase uptake of Aβ in AD patients' macrophages at 2 h and decrease residual Aβ at 24 h after addition of the indicated inhibitor to macrophages. Macrophages were treated by DMSO, 1 μM EEQ, EEQ with TPPU, or cGAS inhibitor H-151 for 2 or 24 h. a) Aβ uptake at 2 h is significantly increased by EEQ+TPPU or H-151 in comparison to DMSO. b) Aβ residual at 24 h is significantly decreased by EEQ+TPPU or H-151 in comparison to DMSO.