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Title
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Journal
Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids, 1869(2)

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Publication Date
2024-03-01

DOI
10.1016/j.bbalip.2023.159432

Peer reviewed

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Author manuscript

Biochim Biophys Acta Mol Cell Biol Lipids. Author manuscript; available in PMC 2025 March 01.

Published in final edited form as:

Biochim Biophys Acta Mol Cell Biol Lipids. 2024 March ; 1869(2): 159432. doi:10.1016/j.bbalip.2023.159432.

Eicosanoid profiles in an arthritis model: Effects of a soluble epoxide hydrolase inhibitor

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Abstract

Rheumatoid arthritis is a common systemic inflammatory autoimmune disease characterized by damage to joints, inflammation and pain. It is driven by an increase of inflammatory cytokines and lipids mediators such as prostaglandins. Epoxides of polyunsaturated fatty acids (PUFAs) are lipid chemical mediators in a group of regulatory compounds termed eicosanoids. These epoxy fatty acids (EpFA) have resolutive functions but are rapidly metabolized by the soluble epoxide hydrolase enzyme (sEH) into the corresponding diols. The pharmacological inhibition of sEH stabilizes EpFA from hydrolysis, improving their half-lives and biological effects. These anti-inflammatory EpFA, are analgesic in neuropathic and inflammatory pain conditions. Nonetheless,

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CONFLICT OF INTEREST
CAT-S and BDH are authors of patents from the University of California the synthesis and application of sEHI for disease treatment. BDH is a founder of EicOsis LLC that has completed phase 2a clinical trials for a sEHI.

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inhibition of sEH on arthritis and the resulting effects on eicosanoids profiles are little explored despite the physiological importance. In this study, we investigated the effect of sEH inhibition on collagen-induced arthritis (CIA) and its impact on the plasma eicosanoid profile. We measured the eicosanoid metabolites by LC– MS/MS-based lipidomic analysis. The treatment with a sEH inhibitor significantly modulated 11 out of 69 eicosanoids, including increased epoxides 12(13)-EpODE, 12(13)-EpOME, 13-oxo-ODE, 15-HEPE, 20-COOH-LTB4 and decreases several diols 15,6-DiHODE, 12,13-DiHOME, 14,15-DiHETrE, 5,6-DiHETrE and 16,17-DiHDPE. Overall the inhibition of sEH in the rheumatoid arthritis model enhanced epoxides generally considered anti-inflammatory or resolutive mediators and decreased several diols with inflammatory features. These findings support the hypothesis that inhibiting the sEH increases systemic EpFA levels, advancing the understanding of the impact of these lipid mediators as therapeutical targets.

Keywords

Lipidomics; soluble epoxide hydrolase; rheumatoid arthritis; diols; inflammation

1. INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory condition affecting the joints, leading to cartilage and bone damage as well as disability [1]. The pathogenesis of RA is multifactorial, involving various contributing factors. Dysregulated cytokine production and altered serum concentrations of several cytokines have been observed in RA patients. Notably, the production and release of specific cytokines such as IL-17A, IL-17F, and IL-22 have been shown to stimulate synovial fibroblasts and macrophages, leading to the secretion of pro-inflammatory mediators including IL-1β, IL-6, TNF-α, and PGE2. These mediators exacerbate synovial inflammation [2]. Furthermore, a recently identified molecule known as secreted osteoclastogenic factor of activated T cells (SOFAT) has been implicated in the complex inflammatory phenotype of RA [3]. These findings highlight the ongoing need for extensive investigations to better comprehend the intricate mechanisms involving the molecules and mediators that initiate and perpetuate RA.

Lipids play crucial roles as essential metabolites, serving as energy sources, components of cell membranes, and signaling molecules, particularly in the context of inflammation [4]. Eicosanoids, in particular, precede the production of cytokines and chemokines [5]. Metabolomic alterations, including changes in the eicosanoid profile, have been implicated in rheumatoid arthritis (RA), leading to a pro-inflammatory response characterized by the release of inflammatory mediators such as TNF-α, IL-17, and IL-4 [6]. Consequently, over the past decade, there has been growing interest in modulating lipid metabolism as a means to better understand the pathogenesis of RA and mitigate tissue damage caused by the inflammatory response [7].

Eicosanoids are derived from the oxidation of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) and are metabolized through various pathways, including cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) pathways [8]. These eicosanoids encompass bioactive molecules such as prostaglandins, lipoxins, and

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leukotrienes, which exert diverse and pleiotropic effects on inflammation and immunity [9]. Within the cytochrome P450 pathway, arachidonic acid (ARA), linoleic acid (LA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) can undergo conversion to epoxy metabolites. These epoxy metabolites, including epoxyeicosatrienoic acids (EETs), epoxyoctadecenoic acids (EpOMEs), and hydroxyeicosapentaenoic acids (HEPEs), have demonstrated anti-inflammatory effects. However, the corresponding diols resulting from the action of the soluble epoxide hydrolase enzyme (sEH) exhibit increased polarity and are easily conjugated for excretion. Notably, some diols have been associated with increased inflammation and pain [10,11]. Consequently, inhibitors targeting the soluble epoxide hydrolase (sEH) enzyme have been developed with the aim of disrupting the enzymatic hydrolysis and activities of various epoxy fatty acids (EpFA). These inhibitors have shown promise in experimental disease treatment, particularly in the control of inflammation [12,13]. In a recent well-executed study, it was elegantly demonstrated that the lipidome profile is significantly influenced by the phase and status of rheumatoid arthritis (RA) disease, enabling discrimination between different phases of disease activity [14].

The soluble epoxide hydrolase (sEH) enzymes are expressed in various tissues including the intestine, liver, kidney, brain, vasculature, spleen, and knee joint [15,16]. Elevated sEH activity and protein presence have been reported in inflammatory diseases such as periodontitis and arthritis [12,16]. In a recent study conducted by our group, we demonstrated that inhibition of sEH leads to a reduction in the inflammatory response in a collagen-induced arthritis model (CIA). This inhibition resulted in decreased levels of pro-inflammatory cytokines associated with Th1 and Th17 profiles, while simultaneously increasing the presence of Treg cells. As a result, hyperalgesia and edema were alleviated [16].

Changes in lipid mediators have been observed even prior to the manifestation of disease, including increased levels of 5-hydroxyeicosatetraenoic acid (5-HETE) [17] and decreased levels of ω–3 fatty acids [18], underscoring the significance of lipid metabolism in rheumatoid arthritis (RA). Consequently, in this study, we investigated the lipidome profile in an arthritis mouse model following inhibition of the soluble epoxide hydrolase (sEH) enzyme.

2. METHODOLOGY

2.1 Animals and animals' care

This study was performed on male mice DBA/1J weighing 25 to 30 g (n = 4/per group), in a total of 12 animals, and kept in cages (4 per cage) in a temperature-controlled room (23 ± 1°C), 12:12 light cycle, with water and food ad libitum. This *in vivo* protocol was performed according to the "NC3Rs ARRIVE Guidelines, Animal Research: Reporting of in Vivo Experiments". All experiments were conducted in accordance with the Committee on Animal Research of Faculdade São Leopoldo Mandic, Brazil (#2019/019), which followed the guidelines by the Brazilian National Council for Control of Animal Experimentation (CONCEA).

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2.2 Collagen-induced Arthritis (CIA) in DBA/1J mice

The methodology used was described previously [19,16] and summarized below. Male DBA/1J mice (6–9 weeks) were immunized intradermally at 1.5 cm from the base of the tail with 100 μg of chicken sternal hyaline Collagen type 2 (CII) (Sigma) dissolved in water with 100 μL acetic acid (0.05 mol/L) and mixed with an equal volume of CFA (complete Freund's adjuvant) (Difco Laboratories, Detroit, MI, USA). After 21 days, animals were boosted with 100 μg CII emulsified in incomplete Freund's adjuvant (Difco). Mice were monitored daily for signs of arthritis as described. Scores were assigned based on erythema, swelling, or loss of function present in each paw on scale of 0–4, giving a maximum score of 16 per mouse (see below for details). When mice reached a score of 1 for clinical arthritis, the animals were randomly assigned to one of the following groups: mice were treated with the sEH inhibitor 1-(1-propanoylpiperidin-4-yl)-3-[4-(trifluoromethoxy)phenyl]urea (TPPU) at a dose of 10 mg/kg by gavage [16] or vehicle (the same volume of PEG400 in saline) daily for 15 days. Scoring was conducted in a blind fashion by an investigator who do not perform the treatment of the animals. At the end of 15 days, all groups were anesthetized with xylazine and ketamine i.p. and sacrificed by cervical dislocation. The blood was collected, and the plasm was separated by centrifugation.

2.3 Clinical arthritis scores

Mice were inspected for the development of CIA and inflammation of the four paws was graded between 0 and 4: 0, paws with no swelling; 1, paws with swelling of finger joints or focal redness; 2, paws with mild swelling of wrist or ankle joints; 3, paws with severe swelling of the entire paw; and 4, paws with deformity or ankylosis. Each paw was graded, and the four scores were added.

2.4 LC–MS/MS-based lipidomic analysis. Extraction and analyses of the regulatory lipid mediator

The extraction process is similar to the protocol as described previously [19]. Briefly, plasma (200 μL) samples were aliquoted to a solution containing methanol and surrogate solution including a deuterated internal standard solution, a mixture of 500 nM of d4 PGF1a, d4 PGE2, d4 TXB2, d4 LTB4, d6 20 HETE, d11 14,15 DiHETrE, d8 9 HODE, d8 5 HETE, and d11 11,12 EpETrE (Cayman Chemical, Ann Arbor, MI). The plasma samples were vortexed and centrifuged prior to solid phase extraction (SPE) with two column volumes of wash solution (5% methanol, 0.1% acetic acid in water) before elution by 0.5 mL of methanol and 1.5 mL of ethyl acetate. The eluents were dried under vacuum and then reconstituted with 50 μL of 200 nM CUDA solution in methanol prior to analysis. The oxylipins were measured on a 1200 SL ultra-high-performance liquid chromatography (UHPLC) (Agilent, Santa Clara, CA) interfaced with a 4000 QTRAP mass spectrometer (Sciex, Redwood City, CA). The separation conditions for LC were optimized to separate the critical pairs of oxylipins, which share the same multiple reaction monitor (MRM) transitions. In brief, separation was achieved on an Agilent Eclipse Plus C18 150×2.1 mm 1.8 μm column with water with 0.1% acetic acid as mobile phase A and acetonitrile/methanol (84/16) with 0.1% acetic acid as mobile phase B. All the parameters on the mass spectrometer were optimized with pure standards (purchased from Cayman Chemical, Ann Arbor, MI) under negative mode. A

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scheduled multiple reaction monitoring (MRM) scan mode was employed to increase the sensitivity of the measurement.

2.5 Statistical analysis

The statistical analyses were performed using Prism 9.2 (GraphPad, La Jolla, CA, USA). The data were first examined for normality using the Kolmogorov-Smirnov test. One-way ANOVA and the post-test of Tukey or nonparametric test Kruskal Wallis followed by Dunn test was used for histopathological scores. $P < .05$ was considered significant. Data are presented as mean ± SD. The metabolomic analysis was carried out utilizing the integrated web-based platform MetaboAnalyst [20].

3. RESULTS

3.1 Clinical score in arthritis

In the past decade, there has been growing recognition of the role of oxylipins in various inflammatory diseases [21], including their association with the progression of rheumatoid arthritis [22,23]. In this study, we aimed to examine the effects of sEH inhibition (sEHI) in an experimental arthritis model using DBA/1J mice. The clinical evaluation was conducted over a period of 15 days following the initial onset of the disease (Figure 1A). Notably, treatment with sEHI resulted in a significant reduction in clinical scores (Figure 1B) compared to the untreated CIA group.

Consequently, we proceeded with the analysis of the plasma oxylipin profile, further advancing our investigation. A total of 69 distinct oxylipins were identified in measurable quantities within the mouse plasma samples. The metabolomic data were normalized and subjected to analysis (Supplementary Figure 1).

3.2 Metabolomic

The enzymatic conversion of polyunsaturated fatty acids (PUFAs) like linoleic acid, arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) results in the generation of biologically active compounds, involving various enzymes including those within the cytochrome P450 family [24]. To investigate the impact of sEH inhibition on different PUFA cascades, we conducted an individual analysis of each PUFA axis.

The sEH enzyme converts EpFAs, which are formed through CYP-catalyzed oxidation, into their corresponding diols. The score plots generated through partial least squares-discriminant analysis (PLS-DA) for the oxylipin data indicate distinct oxylipin profiles for each group (Control, CIA, and CIA+sEHI) (Figures 2A, 3A, 4A, and 5A). Heatmap and clustering analysis of the oxylipin data reveal changes among the groups, as depicted in Figures 2B, 3B, 4B, and 5B. The Variable Importance in Projection (VIP) score is a calculated value that represents the weighted sum of squares of the PLS loadings, taking into account the degree of explained Y-variation in each dimension. VIP scores are computed for each component, and when multiple components are utilized to calculate the feature importance, the average VIP score is determined. Colored boxes on the right side of the figures (Figures 2G, 3I, 4E, and 5D) indicate the relative concentrations of the

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corresponding metabolites, as indicated by the VIP scores for each group (Control, CIA, and CIA+sEHI).

3.3 Effect of sEHI on CYP–derived oxylipins through the arachidonic acid (AA) pathway.

The CYP450 pathway utilizes arachidonic acid (AA) as a substrate for the production of oxylipins through processes such as epoxidation, chain hydroxylation, and ω-hydroxylation. Our data indicates that blocking sEH activity has an impact on CYP-derived oxylipins when comparing the CIA group with the CIA + sEHI group. Specifically, statistically significant reductions were observed in the levels of 14,15-DiHETrE and 5,6-DiHETrE (Figure 2C and D). Conversely, there was a statistically significant increase in 20-COOH-LTB4 (note that LTB4 is a poor substrate for sEH) (Figure 2E). Regarding 11(12)-EpETrE, its concentration was statistically higher in the CIA group compared to the control group ($p < 0.05$), and treatment with sEHI did not alter its plasma levels (Figure 2F). In each pathway (AA, LA, EPA, and DHA), important features were identified through PLS-DA analysis, revealing comparable concentrations of the corresponding metabolites within each group.

3.4 Effect of sEHI on CYP–derived oxylipins through the linoleic acid (LA) pathway.

In terms of the linoleic acid (LA) pathway, notable observations were made. Firstly, there were statistically significant increased levels of (9,10)-EpOME in the CIA group compared to both control and CIA groups ($p < 0.05$) (Figure 3C). Interestingly, significantly higher levels of 12(13)-EpOME were observed in the sEHi-treated group (Figure 3D), while the corresponding diol, 12,13-DiHOME, exhibited a statistically significant decrease in the CIA-treated group compared to both control and CIA groups (Figure 3E). Conversely, the CIA-treated group demonstrated a significant decrease in the diol 15,16-DiHODE when compared to the CIA group (Figure 3F). Additionally, there were significant increases observed in the levels of 13-oxo-ODE and 12(13)-EpODE in the CIA-treated group (Figure 3G and H).

3.5 Effect of sEHI on CYP–derived oxylipins through the Eicosapentaenoic acid (EPA) pathway.

The CYP enzymes utilize eicosapentaenoic acid (EPA) as a substrate to generate fatty acid epoxides (EpETEs), which are subsequently metabolized into dihydroxy fatty acids (DiHETEs) by the sEH enzyme. Our findings indicate that sEH inhibition (sEHI) has an impact on the CYP-derived oxylipins within the EPA pathway. Specifically, our data revealed a significant increase in the concentration of 13-HODE in the CIA group compared to the control group, while the sEHI-treated group exhibited a restoration towards the physiological levels ($p < 0.05$), as illustrated in Figure 4C. Additionally, there was a statistically significant elevation in the concentration of 15-HEPE observed in the sEHI-treated group compared to the CIA group (Figure 4D).

3.6 Effect of sEHI on CYP–derived oxylipins through the Docosahexaenoic Acid (DHA*) pathway.*

The CYP enzymes facilitate the formation of fatty acid epoxides from docosahexaenoic acid (DHA), resulting in the production of epoxides such as 4,5-EpDPE, 7,8-EpDPE,

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and 16,17-EpDPE. Subsequently, these epoxides can be metabolized into diols, including 16,17-dihydroxy-docosapentaenoic acid (16,17-DiHDPE), through the action of the sEH enzyme. In our study, we observed a significant reduction in the levels of 17-DiHDPE in the CIA-treated group compared to the CIA group (Figure 5C).

4. DISCUSSION

Recent studies have demonstrated that the administration of sEH inhibitors (sEHI) improves the clinical symptoms in animal models of arthritis [16,25,26]. However, the effects of sEHI on metabolites of the cytochrome P450 pathway in rheumatoid arthritis (RA) remain poorly understood. Eicosanoids, which are important lipid mediators derived from arachidonic acid (AA) [21], are converted to their corresponding diols by sEH from polyunsaturated fatty acids (PUFAs) in the CYP pathway. In this study, we observed a decrease in two diols from the AA pathway, namely 14,15-DiHETrE and 5,6-DiHETrE (Figure 2C and D). Interestingly, these lipid alterations have been associated with inflammatory diseases. For instance, a study demonstrated that these diols are linked to an increased risk of Alzheimer's disease progression [27]. In another study, elevated levels of 14,15-DiHETrE were observed in the bronchoalveolar lavage of patients with Sarcoidosis, a systemic inflammatory multi-organ disease, and these levels were associated with the CD4/CD8 lymphocyte ratio [28]. Additionally, we identified increased levels of 11(12)-EpETrE, an agonist of peroxisome proliferator-activated receptor gamma (PPARγ) [29,30], exclusively in the CIA group, and treatment with sEHi did not affect its plasma levels (Figure 2F). Furthermore, the omega oxidation product 20-COOH-LTB4, which has been proposed as a biomarker in inflammatory synovial fluids [31], exhibited lower concentrations in line with previous reports, and its levels were increased in the plasma of the sEHi-treated group (Figure 2E). Taken together, these findings suggest that this lipid mediator could be a promising therapeutic candidate warranting further investigation regarding its role in arthritis.

In recent decades, numerous studies have highlighted the diverse protective functions of linoleic acid (LA) in experimental models, including its anti-inflammatory, anticarcinogenic, antiadipogenic, antidiabetic, and antihypertensive properties [32]. More recently, there has been a growing focus on investigating the roles of LA metabolites in various diseases. Specifically, the biological effects of LA metabolites, such as dihydroxy-metabolites (DiHOMEs) and linoleic acid epoxides (such as epoxyoctadecenoic acids [EpOMEs]), have been recognized for their significant involvement in inflammatory diseases and nociception [33,34]. A study demonstrated that the application of sEH inhibition (specifically TPPU) reduces the concentrations of 12,13-DiHOME in nervous tissue and alleviates thermal hyperalgesia in two in vivo models: zymosan-induced and CFA-induced [34]. Furthermore, a recent study indicated that the diols derived from LA could serve as potential biomarkers for severe cases of COVID-19. These diols were found to be more prevalent in severe patients who experienced an intense inflammatory cytokine storm, suggesting their potential role as drivers of the inflammatory response [35].

In the pathway involving linoleic acid (LA), it was observed that treatment with sEH inhibition (sEHi) led to a decrease in the diols 12,13-DiHOME and 15,16-DiHODE (Figure 3E and F). Previous research using the same model has reported that treatment

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with TPPU in animals with CIA (collagen-induced arthritis) results in a reduction in inflammatory pain [16]. In this study, it was found that sEHi treatment reduced the levels of 12,13-DiHOME in the plasma and increased its precursor, 12(13)-EpOME (Figure 3D and E). These findings align with the earlier study's observations of pain reduction [16]. Furthermore, decreased concentrations of EpOMEs and 9,10-DiHOME were also observed in a chronic inflammatory pain study involving the amygdala and the periaqueductal gray [36]. Interestingly, individuals with chronic neck pain have been found to exhibit increased plasma levels of 9,10- and 12,13-DiHOME. This suggests a potential correlation between these diols and pain [37]. Moreover, DiHOMEs have been shown to activate the NF-κB cascade, leading to a pro-inflammatory response and the release of TNFα and Monocyte Chemoattractant Protein 1 (MCP-1) from HL-1 cells [34]. In this study, we demonstrated that the diols from the LA pathway contribute to the advancement of research in the CIA model, where these epoxy or diol compounds could be linked to disease initiation or progression, particularly regarding pain symptoms. Finally, it is worth noting that 13-Oxo-ODE, an endogenous PPAR gamma ligand with anti-inflammatory effects [37], was investigated in plasma, and its levels were found to be upregulated in the sEH inhibition (sEHi) group (Figure 3G). This indicates the resolutive actions of sEH inhibition in the CIA model [16].

The metabolite 15-HEPE, derived from EPA (an omega-3 fatty acid), is known for its anti-inflammatory properties [39,40]. In this study, treatment with the sEH inhibition (sEHi) group resulted in increased levels of 15-HEPE in the plasma (Figure 4D). Additionally, 15-HEPE has demonstrated resolving actions in psoriatic arthritis [41,42]. Conversely, 13-HODE is a lipid mediator that triggers a pro-inflammatory response and contributes to various pathological conditions [43]. It also plays a role in promoting an inflammatory pain state by activating TRPV1 in dorsal root ganglion cell bodies [44]. Considering these findings, it is reasonable to speculate that the observed decrease in levels of 13-HODE in the sEHi group (Figure 4C) could partially explain the previously observed analgesic effect [16].

Concerning the metabolites derived from the DHA pathway (derived from omega-3 fatty acids), it was observed that the levels of 16,17-DiHDPE were lower in the sEH inhibition (sEHi) group compared to the other groups, but there were no changes in the control and CIA groups (Figure 5C). The specific biological effects of 16,17-DiHDPE are not yet well understood; however, it is worth noting that other metabolites within the same group, such as 19,20-DiHDPE, have been found to induce an inflammatory response [45].

A recent study has indicated that inhibiting sEH has a positive impact on the levels of specialized pro-resolving mediators (SPMs) found in saliva during experimental periodontitis [46]. Notably, resolvin E1 and E2, which belong to the EPA and DHA family of bioactive lipid mediators, were among the upregulated compounds resulting from sEH inhibition. Therefore, taking into account these recent findings and the current report, it can be suggested that the protective and resolving effects of sEH inhibition (specifically TPPU) in inflammatory osteolytic conditions are attributed to a shift in the production of bioactive lipids from inflammatory oxylipins (such as eicosanoids) to SPMs and linoleic metabolites.

To summarize, the inhibition of sEH has a notable effect on the oxylipin profile in arthritis. It results in a decrease in several diols, which can trigger an inflammatory response. On the other hand, it leads to an increase in anti-inflammatory and analgesic lipid precursors derived from sEH. These discoveries have the potential to inform the development of novel approaches for treating rheumatoid arthritis (RA) and potentially other inflammatory conditions, as well as enhancing pain management strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

This work was supported in part by the Brazilian funding agency São Paulo Research Foundation (FAPESP) (#2017/22334-; #2019/01151-9; #2019/21176-6); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) - Research Productivity Fellowship to MHN, JTCN; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), the National Institute of Environmental HealthSciences (NIEHS) and NIEHS Superfund Program P42ES04699 and NIEHS River Award IR35 ES0443-1

Data availability

The manuscript has data included as electronic supplementary material and additional data will be made available on reasonable request.

Abbreviations

RA	Rheumatoid arthritis
CIA	collagen induced-arthritis model
PUFA	polyunsaturated fatty acid
COX	cyclooxygenase
LOX	lipoxygenase
P450	Cytochrome P450
AA	Araquidonic acid
LA	linoleic acid
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
sEH	soluble epoxide hydrolase
EET	eicosatrienoic acids
EpOME	epoxyoctadecenoic acid
HEPE	hydroxyeicosapentaenoic acid

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REFERENCES

1. Smolen JS, Aletaha D, McInnes IB, Rheumatoid arthritis, Lancet. 388 (2016) 2023–2038, 10.1016/S0140-6736(16)30173-8 [PubMed: 27156434]
2. Neumann E, Lefevre S, Zimmermann B, Gay S, Muller-Ladner U, Rheumatoid arthritis progression mediated by activated synovial fibroblasts, Trends Mol. Med 16 (2010) 458–468. doi: 10.1016/j.molmed.2010.07.004 [PubMed: 20739221]
3. Napimoga MH, Dantas Formiga WD, Abdalla HB, Trindade-da-Silva CA, Venturin CM, Martinez EF, Rossaneis AC, Verri WA Jr, Clemente-Napimoga JT, Secreted Osteoclastogenic Factor of Activated T Cells (SOFAT) Is Associated With Rheumatoid Arthritis and Joint Pain: Initial Evidences of a New Pathway, Front. Immunol 28 (2020) 1442. doi: 10.3389/fimmu.2020.01442.
4. Han X, Lipidomics for studying metabolism, Nat. Rev. Endocrinol, 12 (2016) 668–679. 10.1038/nrendo.2016.98 [PubMed: 27469345]
5. Wymann MP, Schneiter R, Lipid signalling in disease, Nat. Rev. Mol. Cell. Biol 9 (2008) 162–176. 10.1038/nrm2335 [PubMed: 18216772]
6. Falconer J, Murphy AN, Young SP, Clark AR, Tiziani S, Guma M, Buckley CD, Review: Synovial Cell Metabolism and Chronic Inflammation in Rheumatoid Arthritis, Arthritis & Rheumatol, 70 (2018) 984–999. 10.1002/art.40504
7. Sultana S, Dey R, Bishayi B, Dual neutralization of TNFR-2 and MMP-2 regulates the severity of *S. aureus* induced septic arthritis correlating alteration in the level of interferon gamma and interleukin-10 in terms of TNFR2 blocking, Immunol. Res 66, (2018) 97–119. 10.1007/s12026-0178979-y [PubMed: 29218573]
8. Hoxha M, Zappacosta B, CYP-derived eicosanoids: Implications for rheumatoid arthritis. Prostaglandins Other Lipid Mediat, 146 (2020) 106405. 10.1016/j.prostaglandins.2019.106405
9. Wang Y, Wagner KM, Morisseau C, Hammock BD, Inhibition of the Soluble Epoxide Hydrolase as an Analgesic Strategy: A Review of Preclinical Evidence, J. Pain Res. 14, (2021) 61–72. doi:10.2147/JPR.S241893 [PubMed: 33488116]
10. Tiwari S, Yang J, Morisseau C, Durbin-Johnson B, Hammock BD, Gomes AV, Ibuprofen alters epoxide hydrolase activity and epoxy-oxylipin metabolites associated with different metabolic pathways in murine livers, Sci Rep, 11, (2021) 7042. 10.1038/s41598-021-86284-1 [PubMed: 33782432]
11. McReynolds C, Hammock B, Morisseau C, Regulatory lipid vicinal diols counteract the biological activity of epoxy fatty acids and can act as biomarkers and mechanisms for disease progression, Pharmacol. Ther 248 (2023) 108454. doi: 10.1016/j.pharmthera.2023.108454.
12. Trindade-da-Silva CA, Bettaieb A, Napimoga MH, Lee KSS, Inceoglu B, Ueira-Vieira C, Bruun D, Goswami SK, Haj FG, Hammock BD, Soluble Epoxide Hydrolase Pharmacological Inhibition Decreases Alveolar Bone Loss by Modulating Host Inflammatory Response, RANK-Related Signaling, Endoplasmic Reticulum Stress, and Apoptosis, J. Pharmacol. Exp. Ther, 361 (2017) 408–416. 10.1124/jpet.116.238113 [PubMed: 28356494]
13. Napimoga MH, Rocha EP, Trindade-da-Silva CA, Demasi A, Martinez EF, Macedo CG, Abdalla HB, Bettaieb A, Haj FG, Clemente-Napimoga JT, Inceoglu B, Hammock BD, Soluble epoxide hydrolase inhibitor promotes immunomodulation to inhibit bone resorption. J. Periodontal Res, 53 (2018) 743–749. 10.1111/jre.12559 [PubMed: 29851077]
14. Koh JH, Yoon SJ, Kim M, Cho S, Lim J, Park Y, Kim HS, Kwon SW, Kim WU. Lipidome profile predictive of disease evolution and activity in rheumatoid arthritis. Exp. Mol. Med, 54 (2022) 143–155. 10.1038/s12276-022-00725-z [PubMed: 35169224]
15. Norwood S, Liao J, Hammock BD, Yang GY, Epoxyeicosatrienoic acids and soluble epoxide hydrolase: Potential therapeutic targets for inflammation and its induced carcinogenesis, Am. J. Transl. Res 2 (2010) 447. [PubMed: 20733953]
16. Trindade-da-Silva CA, Clemente-Napimoga JT, Abdalla HB, Rosa SM, Ueira-Vieira C, Morisseau C, Verri WA Jr, Montalli VAM, Hammock BD, Napimoga MH, Soluble epoxide hydrolase inhibitor, TPPU, increases regulatory T cells pathway in an arthritis model, FASEB J, 34 (2020) 9074–9086. 10.1096/fj.202000415R [PubMed: 32400048]

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17. Polinski KJ, A Bemis E, Yang F, Crume T, Demoruelle MK, Feser M, Seifert J, O'Dell JR, Mikuls TR, Weisman MH, Gregersen PK, Keating RM, Buckner J, Reisdorph N, Deane KD, Clare-Salzler M, Holers VM, Norris JM, Association of Lipid Mediators With Development of Future Incident Inflammatory Arthritis in an Anti-Citrullinated Protein Antibody-Positive Population, Arthritis & Rheumatol, 73 (2021) 955–962. 10.1002/art.41631

18. Gan RW, Young KA, O Zerbe G, Demoruelle MK, Weisman MH, Buckner JH, Gregersen PK, Mikuls TR, O'Dell JR, Keating RM, ClareSalzler MJ, Deane KD, Holers VM, Norris JM, Lower omega-3 fatty acids are associated with the presence of anti-cyclic citrullinated peptide autoantibodies in a population at risk for future rheumatoid arthritis: a nested case-control study, Rheumatol 55 (2016) 367–376. 10.1093/rheumatology/kev266

19. Yang J, Schmelzer K, Georgi K, Hammock BD. Quantitative profiling method for oxylipin metabolome by liquid chromatography electrospray ionization tandem mass spectrometry, Anal Chem. 81 (2009) 8085–8093. doi: 10.1021/ac901282n. [PubMed: 19715299]

20. Xia J, Wishart DS, Metabolomic data processing, analysis, and interpretation using MetaboAnalyst, Curr. Protoc. Bioinformatics, 14 (2011) 1–14 doi: 10.1002/0471250953.bi1410s34.

21. Osthues T, Sisignano M, Oxidized Lipids in Persistent Pain States, Front. Pharmacol 15 (2019) 1147. doi: 10.3389/fphar.2019.01147.

22. Łuczaj W, Moniuszko-Malinowska A, Domingues P, Domingues MR, Gindzienska-Sieskiewicz E, Skrzydlewska E, Plasma lipidomic profile signature of rheumatoid arthritis versus Lyme arthritis patients. Arch Biochem Biophys, 654 (2018) 105–114. 10.1016/j.abb.2018.07.021 [PubMed: 30059653]

23. Dorochow E, Köhm M, Hahnefeld L, Gurke R, Metabolic Profiling in Rheumatoid Arthritis, Psoriatic Arthritis, and Psoriasis: Elucidating Pathogenesis, Improving Diagnosis, and Monitoring Disease Activity, J. Pers. Med, 12 (2022) 924. 10.3390/jpm12060924 [PubMed: 35743709]

24. Calder PC, Eicosanoids, Essays Biochem, 64 (2020) 423–441. 10.1042/EBC20190083 [PubMed: 32808658]

25. Teixeira JM, Abdalla HB, Basting RT, Hammock BD, Napimoga MH, Clemente-Napimoga JT, Peripheral soluble epoxide hydrolase inhibition reduces hypernociception and inflammation in albumin-induced arthritis in temporomandibular joint of rats, Int. Immunopharmacol, 87 (2020) 106841. doi: 10.1016/j.intimp.2020.106841.

26. Trindade-da-Silva CA, Clemente-Napimoga JT, Abdalla HB, Basting RT, Napimoga MH. Peroxisome proliferator-activated receptor-gamma (PPARγ) and its immunomodulation function: current understanding and future therapeutic implications, Expert Rev. Clin. Pharmacol, 15 (2022) 295–303. doi: 10.1080/17512433.2022.2071697. [PubMed: 35481412]

27. Morris JK, Piccolo BD, John CS, Green ZD, Thyfault JP, Adams SH. Oxylipin Profiling of Alzheimer's Disease in Nondiabetic and Type 2 Diabetic Elderly, Metabolites, 9(2019) 177. 10.3390/metabo9090177 [PubMed: 31491971]

28. Sjödin MOD, Checa A, Yang M, Dahlén SE, Wheelock AM, Eklund A, Grunewald J, Wheelock CE, Soluble epoxide hydrolase derived lipid mediators are elevated in bronchoalveolar lavage fluid from patients with sarcoidosis: a cross-sectional study, Respir. Res 19, (2018) 236. 10.1186/s12931-018-0939-0 [PubMed: 30509266]

29. Dennis EA, Deems RA, Harkewicz R, Quehenberger O, Brown HA, Milne SB, Myers DS, Glass CK, Hardiman G, Reichart D, Merrill AH, C Sullards M, Wang E, Murphy RC, Raetz CR, Garrett TA, Guan Z, C Ryan A, Russell DW, McDonald JG, Thompson BM, Shaw WA, Sud M, Zhao Y, Gupta S, Maurya MR, Fahy E, Subramaniam S,. A mouse macrophage lipidome, J Biol Chem, 285 (2010), 39976–39985. 10.1074/jbc.M110.182915 [PubMed: 20923771]

30. A Dennis E, Norris PC, Eicosanoid storm in infection and inflammation, Nat. Rev. Immunol, 15 (2015) 511–523. 10.1038/nri3859 [PubMed: 26139350]

31. Costello PB, Baer AN, Green FA, Lipoxygenase products in inflammatory synovial fluids and other exudates, Ann. Rheum. Dis, 51 (1992) 1215–1218. doi: 10.1136/ard.51.11.1215. [PubMed: 1334643]

32. Viladomiu M, Hontecillas R, Bassaganya-Riera J, Modulation of inflammation and immunity by dietary conjugated linoleic acid, Eur J Pharmacol, 785 (2016) 87–95. 10.1016/j.ejphar.2015.03.095 [PubMed: 25987426]

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33. Bergmann CB, McReynolds CB, Wan D, Singh N, Goetzman H, Caldwell CC,Supp DM, Hammock BD, sEH-derived metabolites of linoleic acid drive pathologic inflammation while impairing key innate immune cell function in burn injury, Proc. Natl. Acad. Sci. U. S. A 119 (2022) e2120691119. doi: 10.1073/pnas.2120691119.
34. Zimmer B, Angioni C, Osthues T, Toewe A, Thomas D, Pierre SC, Geisslinger G, Scholich K, Sisignano M. The oxidized linoleic acid metabolite 12,13-DiHOME mediates thermal hyperalgesia during inflammatory pain. Biochim. Biophys. Acta Mol. Cell Biol. Lipids, 1863 (2018), 669–678. doi: 10.1016/j.bbalip.2018.03.012. [PubMed: 29625231]
35. McReynolds CB, Cortes-Puch I, Ravindran R, Khan IH, Hammock BG, Shih PB, Hammock BD, Yang J, Plasma Linoleate Diols Are Potential Biomarkers for Severe COVID-19 Infections, Front Physiol, 12 (2021) 663869. 10.3389/fphys.2021.663869
36. Jensen JR, Pitcher MH, Yuan ZX, Ramsden CE, Domenichiello AF, Concentrations of oxidized linoleic acid derived lipid mediators in the amygdala and periaqueductal grey are reduced in a mouse model of chronic inflammatory pain, Prostaglandins Leukot. Essent. Fatty Acids, 135 (2018) 128–136. doi: 10.1016/j.plefa.2018.07.015. [PubMed: 30103924]
37. Hellström A, Ley D, Hansen-Pupp I, Hallberg B, Löfqvist C, van Marter L, van Weissenbruch M, Ramenghi LA, Beardsall K, Dunger D, Hård AL, Smith LE, Insulin-like growth factor 1 has multisystem effects on foetal and preterm infant development, Acta Paediatr, 105 (2016) 576–586. 10.1111/apa.13350 [PubMed: 26833743]
38. Altmann R, Hausmann M, Spöttl T, Gruber M, Bull AW, Menzel K, Vogl D, Herfarth H, Schölmerich J, Falk W, Rogler G, 13-Oxo-ODE is an endogenous ligand for PPARgamma in human colonic epithelial cells, Biochem Pharmacol, 74 (2007) 612–622. 10.1016/j.bcp.2007.05.027 [PubMed: 17604003]
39. Sawane K, Nagatake T, Hosomi K, Hirata SI, Adachi J, Abe Y, Isoyama J, Suzuki H, Matsunaga A, Fukumitsu S, Aida K, Tomonaga T, Arita M, Kunisawa J, Dietary Omega-3 Fatty Acid Dampens Allergic Rhinitis via Eosinophilic Production of the Anti-Allergic Lipid Mediator 15-Hydroxyeicosapentaenoic Acid in Mice, Nutrients, 11 (2019), 2868. 10.3390/nu11122868 [PubMed: 31766714]
40. Rohwer N, Chiu CY, Huang D, Smyl C, Rothe M, Rund KM, Helge Schebb N, Kühn H, Weylandt KH, Omega-3 fatty acids protect from colitis via an Alox15-derived eicosanoid. FASEB J, 35 (2021) e21491. 10.1096/fj.202002340RR
41. Coras R, Kavanaugh A, Boyd T, Huynh Q, Pedersen B, Armando AM, Dahlberg-Wright S, Marsal S, Jain M, Paravar T, Quehenberger O, Guma M, Pro- and anti-inflammatory eicosanoids in psoriatic arthritis, Metabolomics, 15 (2019) 65. 10.1007/s11306-019-1527-0 [PubMed: 31004236]
42. Coras R, Kavanaugh A, Kluzniak A, Holt D, Weilgosz A, Aaron A, Quehenberger O, Ritchlin C, Guma M, Differences in oxylipin profile in psoriasis versus psoriatic arthritis, Arthritis Res Ther, 23 (2021) 200. 10.1186/s13075-021-02575-y [PubMed: 34303373]
43. Pecorelli A, Cervellati C, Cordone V, Amicarelli F, Hayek J, Valacchi G, 13-HODE, 9-HODE and ALOX15 as potential players in Rett syndrome OxInflammation, Free Radic. Biol. Med, 134 (2019) 598–603. 10.1016/j.freeradbiomed.2019.02.007 [PubMed: 30743046]
44. Alsalem M, Wong A, Millns P, Arya PH, Chan MS, Bennett A, Barrett DA, Chapman V, Kendall DA, The contribution of the endogenous TRPV1 ligands 9-HODE and 13-HODE to nociceptive processing and their role in peripheral inflammatory pain mechanisms, Br J Pharmacol, 168 (2013), 1961–1974. 10.1111/bph.1209 [PubMed: 23278358]
45. Bruins MJ, Dane AD, Strassburg K, Vreeken RJ, Newman JW, Salem N Jr, Tyburczy C, Brenna JT, Plasma oxylipin profiling identifies polyunsaturated vicinal diols as responsive to arachidonic acid and docosahexaenoic acid intake in growing piglets, J. Lipid. Res, 54 (2013) 1598–1607. doi: 10.1194/jlr.M034918. [PubMed: 23543770]
46. Abdalla HB, Alvarez C, Wu YC, Rojas P, Hammock BD, Maddipati KR, Trindade-da-Silva CA, Soares MQS, Clemente-Napimoga JT, Kantarci A, Napimoga MH, Van Dyke TE, Soluble epoxide hydrolase inhibition enhances production of specialized pro-resolving lipid mediator and promotes macrophage plasticity, Br. J. Pharmacol, 180 (2023) 1597–1615. doi: 10.1111/bph.16009 [PubMed: 36508312]

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Highlights

Rheumatoid arthritis is a disease characterized by joint damage, inflammation, and pain.

Epoxides of polyunsaturated fatty acids (PUFAs) play a role as lipid chemical mediators

Preventing rapid metabolism into diols is achieved by inhibiting soluble epoxide hydrolase (sEH).

The impact of sEH inhibition on plasma eicosanoid profile was assessed in CIA.

sEH inhibition modulated eicosanoids, increasing anti-inflammatory epoxides and reducing inflammatory diols.

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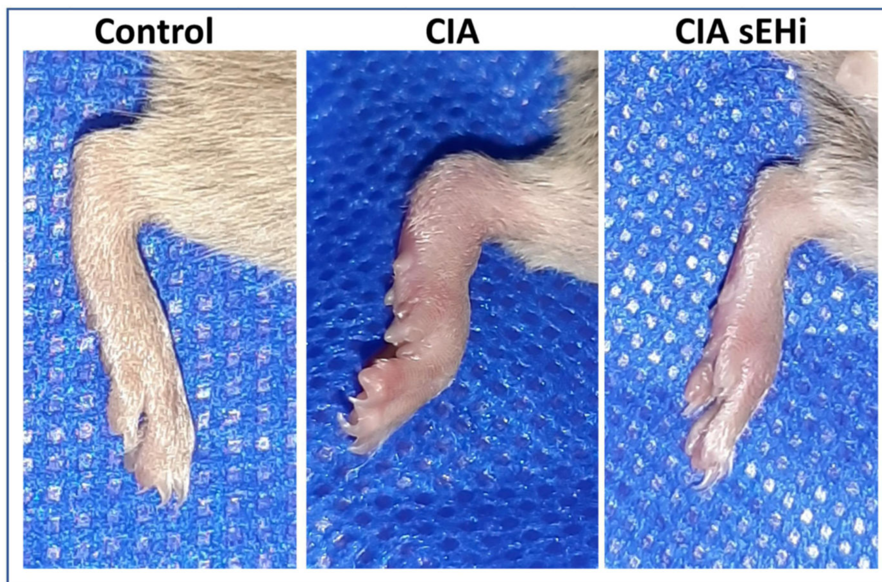

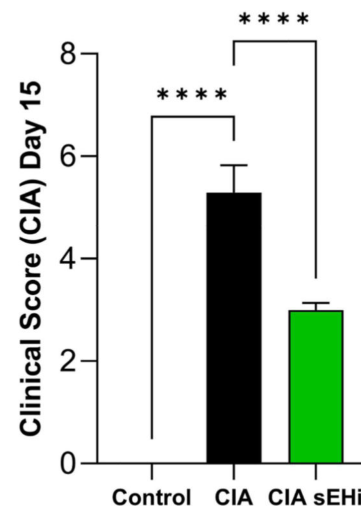

Figure 1:

Amelioration of clinical score in arthritis model by soluble epoxide hydrolase inhibitor. Oral treatment with TPPU (10mg/kg) was given daily for 15 days in the CIA + sEHI group. Disease progression was monitored (A) and quantified using clinical scores (B). The results are presented as mean ± SD, with n = 4 mice per group in each experiment. Statistical analysis was performed using two-way ANOVA followed by Tukey's post-test, with **** indicating $p < 0.0001$.

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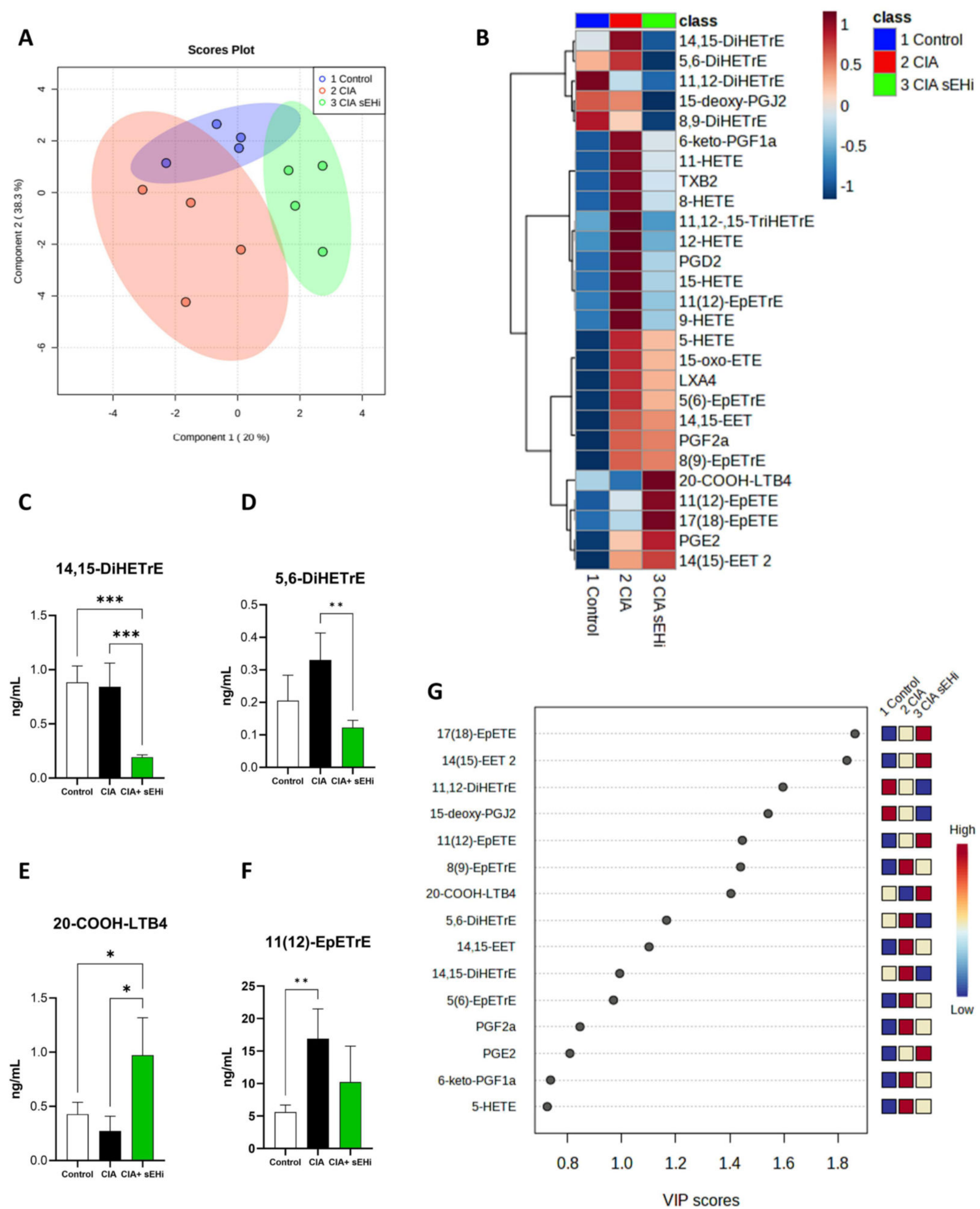

Figure 2.

Profile of arachidonic acid metabolites upon treatment with sEHI in an arthritis model. Oral treatment with TPPU (10mg/kg) was given daily for 15 days in the CIA + sEHI group. (A) Score plots of Partial Least Squares-Discriminant Analysis (PLS-DA) illustrating the differentiation of Control, CIA, and CIA + sEHI groups. (B) Heatmap and clustering analysis displaying the relative intensities of eicosanoid variables in the control, CIA, and CIA + sEHI groups, represented by color bars. Column bar graphs demonstrating changes in the levels of CYP-derived oxylipins from arachidonic acid (AA) including:

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(C) 14,15-DiHETrE, (D) 5,6-DiHETrE, (E) 20-COOH-LTB4, and (F) 11(12)-EpETrE. (G) Identification of significant features by PLS-DA. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group (Control, CIA, or CIA + sEHI). Values are presented as mean ± SE; n = 4 per group. Statistical significance is denoted by *$p < 0.05$, **$p < 0.001$, and ***$p < 0.0001$.

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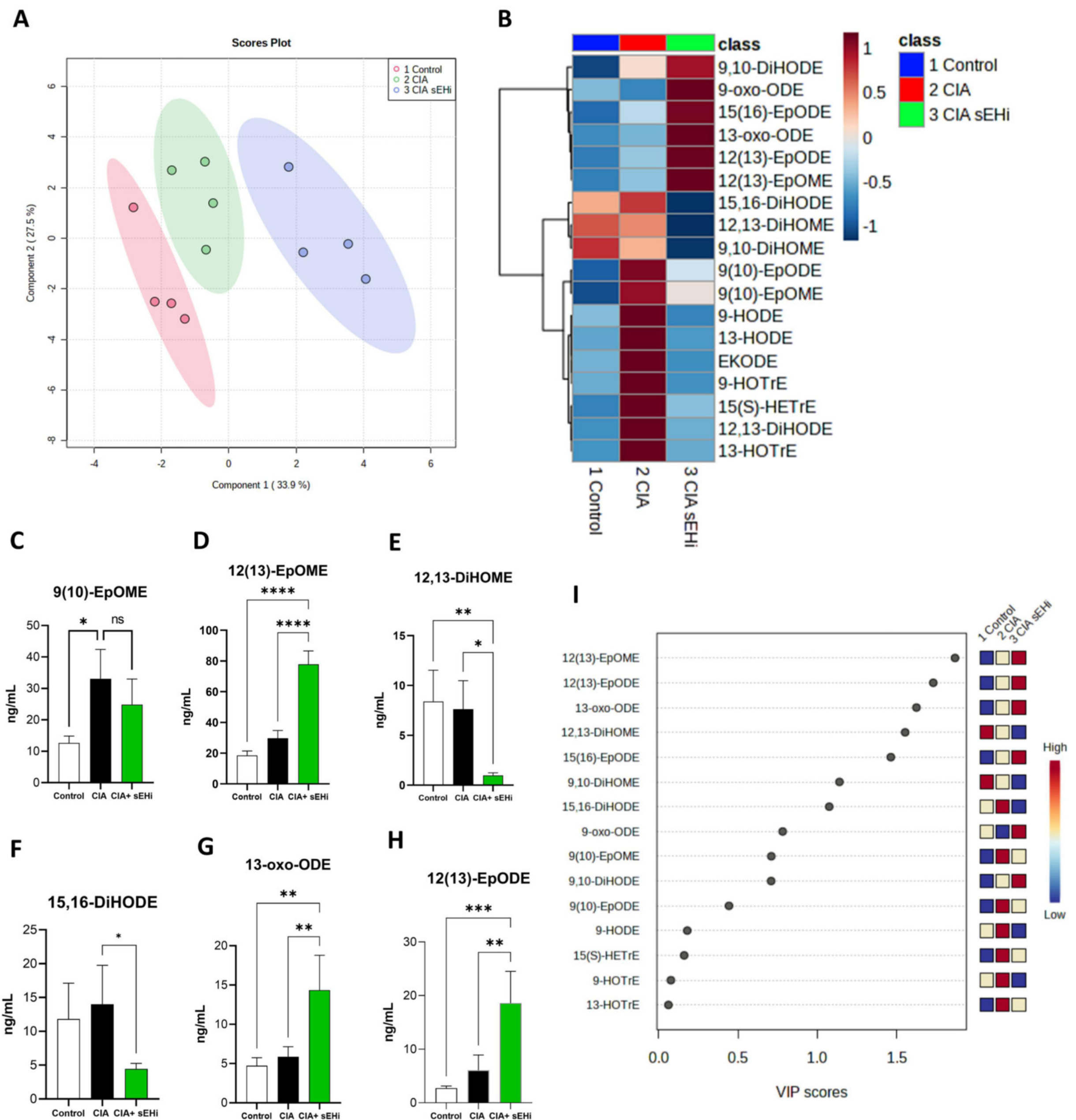

Figure 3:

Profile of linoleic acid metabolites upon treatment with sEHI in an arthritis model. Oral treatment with TPPU (10mg/kg) was given daily for 15 days in the CIA + sEHI group. (A) Score plots of Partial Least Squares-Discriminant Analysis (PLS-DA) illustrating the differentiation of Control, CIA, and CIA + sEHI groups (n = 4 per group). (B) Heatmap depicting the relative intensities of eicosanoid variables in the control, CIA, and CIA + sEHI groups, represented by color bars. Column bar graphs demonstrating changes in the levels of CYP-derived oxylipins from Linoleic Acid including: (C) 9,10-EpOME,

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(D) 12(13)-EpOME, (E) 12,13-DiHOME, (F) 15,16-DiHODE, (G) 13-oxo-ODE, and (H) 12(13)-EpODE. (I) Identification of significant features by PLS-DA. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group (Control, CIA, and CIA + sEHI). Values are presented as mean ± SE; $n = 4$ per group. Statistical significance is denoted by *$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$, and ****$p < 0.00001$.

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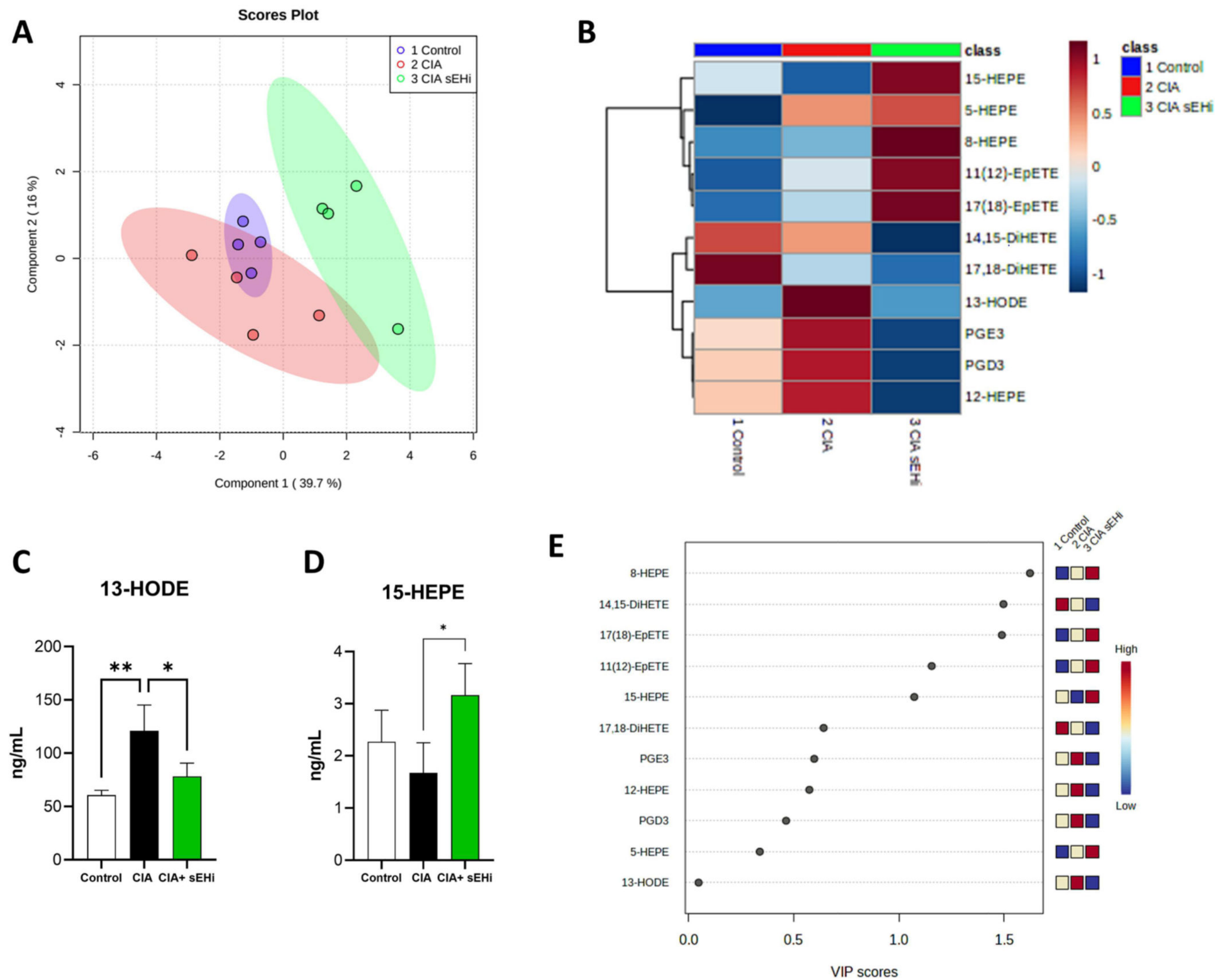

Figure 4:

Profile of eicosapentaenoic acid (EPA) metabolites upon treatment with sEHI in an arthritis model. Oral treatment with TPPU (10mg/kg) was given daily for 15 days in the CIA + sEHI group. (A) Score plots of Partial Least Squares-Discriminant Analysis (PLS-DA) illustrating the differentiation of Control, CIA, and CIA + sEHI groups (n = 4 per group). (B) Heatmap displaying the relative intensities of eicosanoid variables in the control, CIA, and CIA + sEHI groups, represented by color bars. (C) Column bar graphs depicting the changes in the levels of CYP-derived oxylipins from EPA, including 13-HODE. (D) Column bar graph showing the change in the level of 15-HEPE. Values are presented as mean ± SE; n = 4 per group. Statistical significance is denoted by *p < 0.05 and **p < 0.001. (G) Identification of important features by PLS-DA. The colored boxes on the right indicate the relative

concentrations of the corresponding metabolite in each group (Control, CIA, and CIA + sEHI).

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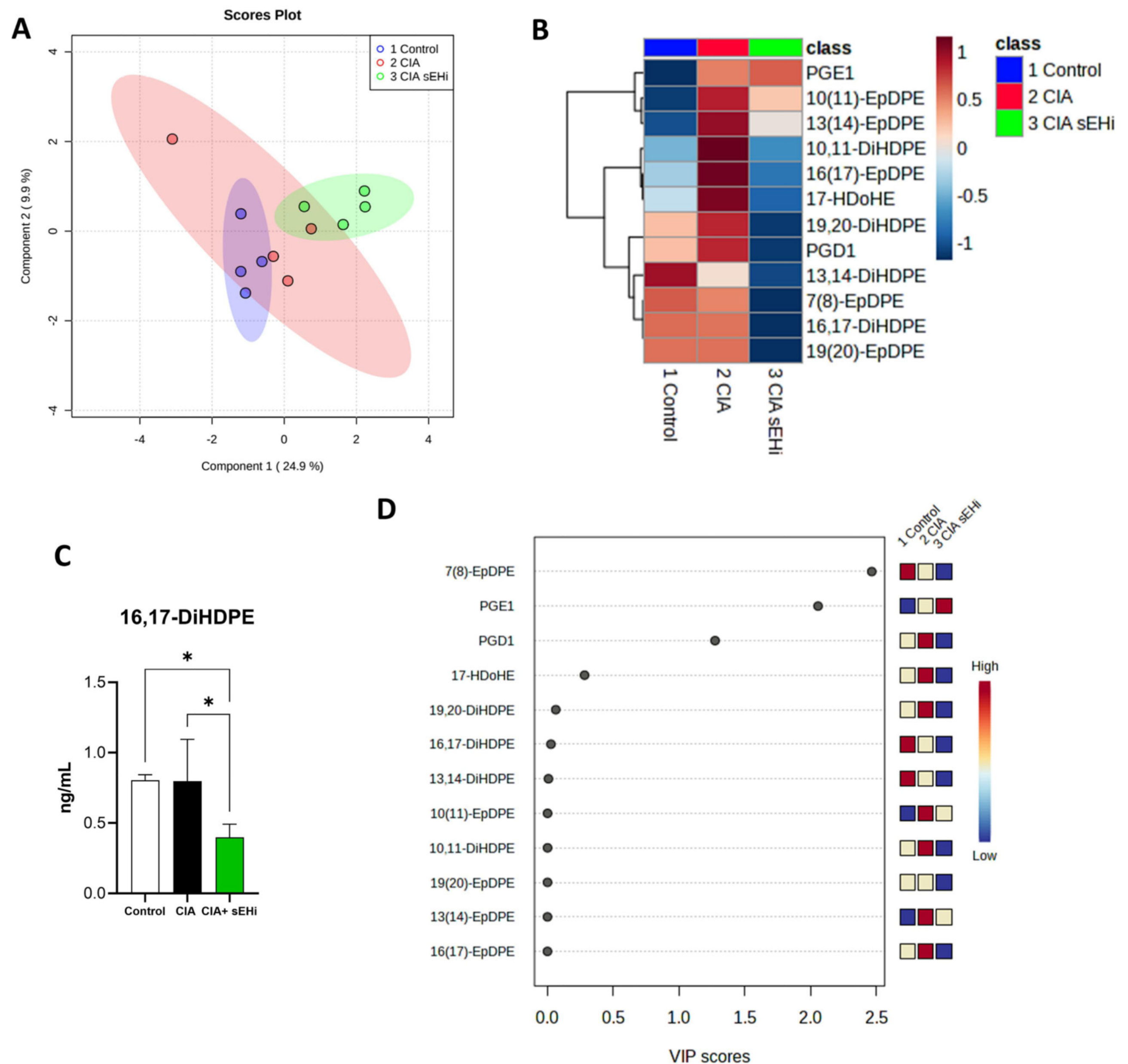

Figure 5:

Profile of docosahexaenoic acid (DHA) metabolites upon treatment with sEHI in an arthritis model. Oral treatment with TPPU (10mg/kg) was given daily for 15 days in the CIA + sEHI group. (A) Score plots of Partial Least Squares-Discriminant Analysis (PLS-DA) illustrating the differentiation of Control, CIA, and CIA + sEHI groups (n = 4 per group). (B) Heatmap displaying the relative intensities of eicosanoid variables in the control, CIA, and CIA + sEHI groups, represented by color bars. Column bar graph showing the change in the levels of CYP-derived oxylipins from DHA, including (C) 16,17-DiHDPE. Values are presented

as mean ± SE; n = 4 per group. Statistical significance is denoted by *$p < 0.05$. (D) Identification of important features by PLS-DA. The colored boxes on the right indicate the relative concentrations of the corresponding metabolite in each group (Control, CIA, and CIA + sEHI).

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